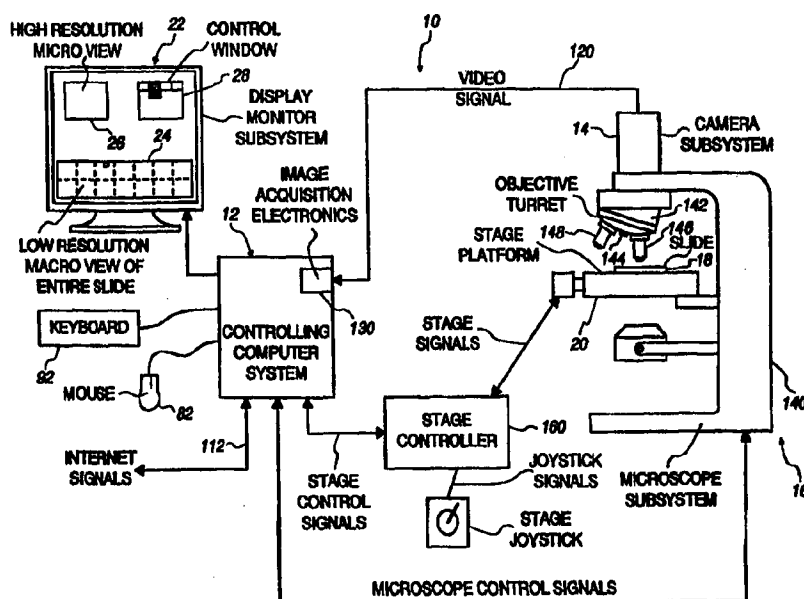




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b>  <b>G06K 9/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/44446</b>  <b>(43) International Publication Date:</b> 8 October 1998 (08.10.98)
<b>(21) International Application Number:</b> PCT/US98/04208  <b>(22) International Filing Date:</b> 2 March 1998 (02.03.98)  <b>(30) Priority Data:</b> 08/805,856      3 March 1997 (03.03.97)      US Not furnished      27 February 1998 (27.02.98)      US  <b>(71) Applicant:</b> BACUS RESEARCH LABORATORIES, INC. [US/US]; Suite 8A, 910 Riverside Drive, Elmhurst, IL 60126 (US).  <b>(72) Inventors:</b> BACUS, James, V.; 4324 Stonewall, Downers Grove, IL 60515 (US). BACUS, James, W.; 20 Natoma Drive, Oakbrook, IL 60521 (US).  <b>(74) Agents:</b> SAMPLES, Kenneth, H. et al.; Fitch, Even, Tabin & Flannery, Room 900, 135 South LaSalle Street, Chicago, IL 60603 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>

**(54) Title:** METHOD AND APPARATUS FOR ACQUIRING AND RECONSTRUCTING MAGNIFIED SPECIMEN IMAGES FROM A COMPUTER-CONTROLLED MICROSCOPE

**(57) Abstract**

A computer-controlled microscope (16) captures a plurality of images at a low magnification. These images are tiled to create a reconstructed image (24) of an entire specimen. The user selects one or more areas of the reconstructed image. The computer-controlled microscope (16) then captures a plurality of high resolution images (26) of the selected areas for display to the user.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon	KR	Republic of Korea	PL	Poland		
CN	China	KZ	Kazakhstan	PT	Portugal		
CU	Cuba	LC	Saint Lucia	RO	Romania		
CZ	Czech Republic	LI	Liechtenstein	RU	Russian Federation		
DE	Germany	LK	Sri Lanka	SD	Sudan		
DK	Denmark	LR	Liberia	SE	Sweden		
EE	Estonia			SG	Singapore		

-1-

**METHOD AND APPARATUS FOR ACQUIRING  
AND RECONSTRUCTING MAGNIFIED SPECIMEN IMAGES  
FROM A COMPUTER-CONTROLLED MICROSCOPE**

**Field of the Invention**

5           This invention relates to a method of and  
apparatus for acquiring and recording digital images of  
an optical image viewed through a computer-controlled  
automated microscope and also, to using the latter in a  
quantitative analysis of plant or biological specimens.

**Background of the Invention**

10           In the image analysis and quantification of DNA  
from tissue sections as disclosed in United States Patent  
4,741,031, and also especially in the immunohisto-  
chemistry assays on the kinds of cell analysis systems  
15 disclosed in United States Patents 5,086,476; 5,202,931;  
and 5,252,487 issued to Bacus, there is a problem of  
first locating the cancer regions for analysis under low  
power and then remembering them when performing the  
analysis under higher power. There is a need, a  
20 requirement to image and digitally record an object in a  
relatively flat plane at high resolution/magnification.  
Today, it is impractical to construct an optical image  
sensor large enough to cover the entire image area e.g.,  
of a specimen on a microscope slide, at the required  
25 resolution. This is because lens size and  
resolution/magnification issues limit the size of the  
field of view of magnified objects and their resulting  
images. Viewing through a microscope is akin to viewing  
through a periscope in that one sees a very small field  
30 of view even at low magnifications, such as 1.25X. A  
pathologist using a microscope often scans a slide to  
obtain in his mind an overall view or sense of what  
constitutes the specimen and he remembers the general  
locations of the diagnostically significant, small pieces  
35 of the specimen. Usually, these are the diseased areas,  
such as malignant or potentially malignant portions of

-2-

the specimen. To obtain higher resolution and magnification of these suspicious portions, the pathologist switches to a higher magnification objective lens but then the field of view becomes much smaller again. Often, the pathologist switches back and forth between the lower magnification, larger field of view objective lens to orient himself relative to the specimen and the high magnification, smaller field of view to obtain the detailed, high resolution view of the suspicious area on the specimen. Thus, the user never receives a magnified, condensed overall view of the specimen or a portion of the specimen but must remember the series of views taken at low magnification. Likewise, at high resolutions and high magnifications, the user never receives or views a collection of adjacent images but must interrelate these successive images in the user's mind.

Stated differently, the problem is that once the microscope is set up for quantitation by image analysis under, e.g. 40x, where all of the diaphragms are set and light adjusted, etc., if the operator needs to move to another tissue area, it is first desirable to locate it at e.g. 10x. In fact, often regions can only be located at this power. In order to do so however, all of the settings (diaphragms, light levels, wavelengths of light, etc.) have to be changed to view the tissue at this magnification. Currently, there is no way to ensure that one could go back to the settings at the previous 40x magnification and continue on with the quantitative image analysis of that same specimen. This necessitates finding those areas under 40x, without changing objectives, which is very slow and time-consuming, and often important cancer areas can be missed.

Also, another problem with tissue analysis, at its current state-of-the-art, is that it is not completely automated, for example, with regard to finding structural regions such as glands, basal layers or other

-3-

important diagnostic regions. However, as set forth in my co-pending patent application Serial No. 701,974, filed August 23, 1996, if these regions are located, important very sensitive diagnostic measurements can be performed, which patent application is incorporated by reference as if fully reproduced herein. For example, as disclosed in the aforesaid patent application, assays are made of a variety of tissue types, both human and animal for analysis of neoplasia in tissue, for pre-invasive cancer in tissue, and the effects on the tissue of chemopreventive agents. A quantitative analysis by image processing techniques is performed on tissue types, having various architectural features, such as breast tissue, colon tissue, prostate tissue, esophageal tissue, skin tissue, cervix tissue, etc. These tissues have different morphologies, and they undergo different neoplasias usually resulting from a cellular mutation, as may be enhanced by a carcinogen, or resulting from a cellular proliferation rate enhanced by hormones, growth factors, or other inducers of abnormal tissue growth. Often it is desired to quantify small changes in the neoplasia when it is incipient or through a series of analyses performed at close time intervals to measure whether the neoplasia progression is increasing or has been slowed, stopped or regressed.

Usually, the tissue specimens are cut to expose the basal layer for review under the microscope. Typically, the quantitative measurements are performed at 40x to obtain 100 to 400 tissue images. The 40x objective provides a narrow field of view of a very small portion of the entire basal layer. Often, the basal layer is somewhat elongated and generally linear such as a basal layer in a rat esophagus; and the analysis of the basal layer requires examining it along its length. The basal layer in a mouse colon is more in the form of an irregular, circular shape; and the analysis of this basal layer requires traveling about this circular shape. In

-4-

breast tissue samples, suspected tumor areas may be at widely-spaced points in the stained tissue; and one wants to be able to navigate and travel to these specific suspected areas and to do the 40x analysis at these areas  
5 in an efficient manner. There is a need to allow an experienced operator to interact with the analysis to locate and identify such regions in an interactive manner. Especially, an interactive manner that would be familiar and consistent with the use of a microscope  
10 manually, with high power magnification and low power magnification simultaneously available, but performed on a computer terminal. Such a level of interaction is different than the interaction with the system disclosed in the above-listed Bacus Patents. There is a need to  
15 take the level of interaction to a higher level and let each component, the human and the computer, perform the part that it does best, in the most cost-effective manner.

The current state of archiving the digital  
20 images achieved through a microscope is often by having photographs or by video tapes. The photographs are difficult to use as is a video tape particularly when the user wants to move rapidly back and forth between various images and to scroll through various adjacent parts of  
25 the specimen image. Further, current archival methods lack an overall macro image of the specimen, which allows the user to know exactly where the particular high resolution view is from when the user is making an analysis of the high resolution image.

30 While digitized images can be stored magnetically or otherwise digitized and recorded on various recording mediums, no current archival system allows the user to toggle back and forth between high magnification images and low magnification images or  
35 between various images at different magnifications such as that achieved by a pathologist switching microscope objective lenses in real time to get the macro and micro

-5-

images from the same location on the specimen.

Heretofore, the practice of pathology has been relatively limited to the use of microscopes and to the pathologist having to use the microscope to review the particular

5 specimen.

There is a need for a dynamic system whereby one or more or several pathologists, including a consulting pathologist, may view the same area simultaneously and interact with one another either in diagnosis or in  
10 analysis. Also, it would be best if the images from the specimen could be stored so that a pathologist could easily examine the images at his leisure using an intranet or Internet browser at a later date merely by accessing the particular web site where the images are  
15 located.

A similar problem exists on the Internet or intranet where a pathologist may receive a single field of view magnified image taken from a specimen over the Internet or the intranet on his browser. The pathologist  
20 must be provided with explanations to coordinate the high resolution view with the lower resolution view. The number of views available to the pathologist is very limited, and the pathologist is unable to select other views or to scroll to neighboring views at the areas that  
25 are most interesting to the pathologist.

There are available on the market computer-controlled, automated microscopes such as those sold by Carl Zeiss, Inc., Thornwood, N.J., under the name Axioplan 2 for taking photographic images of a specimen  
30 in the microscopic field of view. Those particular microscopes have computer-controlled and automatically adjusted subsystems, such as an illumination subsystem, a focusing subsystem, a diaphragm or optical stops subsystem, an objective lens subsystem, or a filtering  
35 subsystem. As an operator selects changes from one objective lens, such as one providing low magnification, e.g., 4x, to a higher magnification, e.g., 40x, the

-6-

computer-automated system will turn the lens turret to switch in the high magnification automatically and adjusts the lens and also automatically adjusts the illumination to eliminate glare and to provide the proper light illumination including light density. Further, the focus is adjusted, and the proper diaphragm openings are automatically reset. Thus, the computer-controlled, automated subsystems automatically reset to values stored and predetermined for each selected objective lens and the analysis being done.

Those particular microscopes can be used to view various objects or specimens, but are most typically used to view and to take still photographs of biological specimens, such as tissues and cells. Those particular microscopes lack a computer-controlled X and Y stage for translating a specimen-carrying slide with respect to the field of view of the selected objective lens. Currently, pathologists and others who use such microscopes want to view the specimen images in full color or in enhanced colors using fluorescent illumination and/or monochromatic images using the automated filter subsystems on the microscopes. Currently trained pathologists or clinicians are accustomed to manually adjust and have a microscope available to them to view larger areas of the specimen at low magnification, and then to momentarily switch in a new higher magnification lens to obtain a more highly magnified image of a portion of the specimen viewed at low magnification.

Pathologists and those working in this area have created in themselves a desire to view suspect tissue as though they were viewing the specimen tissue through a microscope and appear to resist analysis systems that do not provide them this ability.

The microscopic field of view reduces very substantially as the magnification increases. The skill level of the clinician and/or pathologist is important to locate viewing the most suspicious areas or points of



-7-

interest on the specimen. Sometimes, a technician will do a first assay and analysis. A pathologist will return to the selected points of interest or other points of interest for review and analysis. One concern with  
5 respect to a quantitative analysis of breast cancer tissue or prostate biopsy tissue samples to pap smears or other tests for various cancers or the like is that a particularly suspicious point in the tissue may be overlooked and missed during the visual assay or for  
10 selection for an automated review analysis. When observing at high magnifications, the field of view is limited to very small area of the specimen. Hence, the observer has difficulty in knowing and remembering the actual, exact location of this small periscopic view  
15 within the very large whole specimen.

Often, also the problem is finding or locating the tissue or cells for view at high magnification so that artifacts and/or blank spaces on the slide are not viewed. A number of approaches have been proposed to  
20 prescreen and locate by an X and Y address the cells or small points of interest from a very large number of potential points of interest.

There are currently available commercial services for prescreening pap smears where one can mail  
25 in slides and the service will do a microscopic prescan at high magnification for suspected or suspicious areas of interest which are marked and given address locations, and also a video tape of the slide specimen is returned by this service to the sender. The sender then reviews  
30 the areas of interest located during the prescreening and/or the video tape to complete the analysis.

In an attempt to locate and allow review of specified points of interest, U.S. Patent No. 5,428,690 to Bacus discloses a system for prescreening of a field  
35 of cells on a specimen slide at low magnification before the viewer. When seeing a point of interest to be viewed at high magnification, the viewer will operate a switch

-8-

or the like to select and record the address of these selected prescreened points of interest. Later, these prescreened points of interest are then brought into position to be analyzed at high magnification. This  
5 system is fairly or too slow for many uses.

A very expensive system is currently in use in which a pathologist located at a diagnostic center is able to make a diagnostic opinion with respect to specimens under a microscope at a remote center. The  
10 pathologist at the diagnostic center manipulates robotic controls to send telepathy signals via a special, dedicated satellite or other large bandwidth channel to control the microscope at the remote site in approximately real time. The pathologist then can  
15 maneuver the remote microscope to shift the microscope's field of view and to send, by telepathy, a highly magnified, very small image back to the pathologist. This system requires each subscriber to have a special microscope operable by manipulation of the robotic  
20 controls at the diagnostic center and a dedicated or large bandwidth channel to convey real time video signals and hence results in a very high cost for the assay being done. To assist the pathologist in staying within the specimen at the remote site, a peripheral edge or map of  
25 the specimen is made using a second video camera and a light box or by using computerized scanning equipment to trace the outline or peripheral edge of the specimen boundaries. A small circle of light is displayed within the map of the specimen so that the pathologist at the  
30 diagnostic center knows the location of the field of view of the highly magnified image within the specimen. In a sense, the pathologist is operating in real time in the same way that he would use his own microscope at his diagnostic center except for a slight transmission delay  
35 used to transmit the video signals of the highly magnified image over large bandwidth channel. Although the pathologist has a small map or peripheral outline of

-9-

the specimen, the pathologist's field of view of the actual specimen is only the small circle of view that is coming through the microscope objective lens. This does not help the pathologist locate suspicious areas of interest as in a prescreening of the entire tissue. The pathologist may switch to the lowest magnification to get the largest field of view of a small section of the specimen, but he never views the entire specimen at any magnification. Also, there is no image analysis quantitative testing from the received images at the diagnostic center; and no quantitative assaying is done with these images at the diagnostic center.

There is a particular interest today in using the Internet system because it is so readily accessible by users at a low cost and using a computer and viewing screen connected to the computer. One problem with trying to do any transmission of digitized, microscopic, highly magnified images over the Internet is that the bandwidth is too narrow to accommodate the tremendous amount of stored data which needs to be transmitted. There is a need for a system which would allow a pathologist or another person, to be able to perform tissue analysis or quantitative assays using a standard computer terminal from a location remote from the automated microscope system that digitized the optical images.

#### Summary of the Invention

In accordance with the present invention, a person such as a pathologist at a computer terminal may view on a computer screen or monitor digitized images of a microscope specimen at different magnifications as selected by the person. Further, the person may receive on the screen a low magnification, reconstructed image of the entire specimen to aid the person in interactively selecting points of interest on the specimen, such as along a basal layer of a tissue specimen.

-10-

More specifically, and in accordance with the present invention, the microscope's small field of view limitation of a specimen is overcome by providing to the viewer a reconstructed, digitized and magnified image of the entire specimen (or a very large portion of the specimen) for performing a visual analysis of the entire tissue in full color to aid in the selection of points of interest to be viewed at a higher magnification and higher resolution. This is achieved by acquiring and storing a large number of low magnification images of the specimen through a microscopic scanning system, e.g., 35 image tiles of the specimen at 1.25X, and then assembling and coordinating these stored tiled images to form an overall, low magnified image of the specimen, i.e., a macro image of the specimen. Preferably, the digitized, stored macro image is reduced in size by a software system to a smaller size, e.g., a 1/4 size image and it is this reduced in size macro tiled image that is displayed on a local screen or is sent over a low band width or a high band width channel to a remote screen. The pathologist not only does not need to have others do a slow laborious prescreening to locate suspicious areas for analysis or for viewing at high magnification, he can use his own experiences to enable him to go directly to the most suspicious areas which he sees on the macro image. He can, on a priority basis, do the most suspicious area first, followed by lower priority areas of interest.

In accordance with the present invention, there is provided a new and improved microscopic display system that displays the low magnification composite image of the specimen to allow the user to view and to interactively select points of interest, each of which may be displayed at high magnification and resolution. This is achieved by providing the user with a marker, such as a cursor or the like, to select the defined area of interest from the stored, macro image; and to acquire

-11-

reproduced, spatially adjacent high resolution, digitized images of the selected area of interest. More specifically, the specimen, when it was first scanned at low magnification to provide a macro view of the specimen, the addresses or locations of the tile images and/or pixels for the composite image were acquired. Therefore, any selected region of interest in the macro image has locations to which the microscopic stage may be automatically repositioned under a changed, higher magnification lens to acquire higher magnification, digitized image tiles that can be assembled into a micro image. Herein, both the macro and micro images are formed of adjacent digitized image tiles which have been coordinated to reproduce spatially the original image that was scanned.

It is the high magnification images, usually at 40x, that were analyzed using image processing techniques as disclosed in the aforesaid patent application, to provide an assay or numerical histological data for the specimen.

In accordance with the preferred embodiment of the invention, the pathologist may desire to see a larger region for analysis at high resolution than can be accommodated at this magnification on his high magnification viewing screen. The pathologist can quickly change the view on his screen to view adjacent, highly magnified, digitized image tiles at this high resolution by scrolling up or down or right to left to shift these digitized, adjacent image tiles into view on the screen. Thus, even at a higher magnification of a region the pathologist is able to obtain a much larger view than the small field for the objective lens in use of adjacent tissue or cells to give him a broader, overall perspective of what is happening or what has happened in specific section of a specimen. For instance, a pathologist may want to see at high magnification and to assay at this high magnification, a

-12-

suspicious area, the pathologist can draw a mark about the area and cause it to then be assayed and displayed.

By having displayed on a low magnification screen or split screen of the full composite area, by  
5 having the high magnification region being marked on the low image screen, and by having the region being scrolled to view adjacent high magnification images on the high magnification screen, the pathologist has available information to guide him in the sense of helping to  
10 navigate intelligently within the specimen in his search for cancerous tissue or the like for further inspection or measurement of malignant characteristics. Often, when restricted to a field of view of an objective microscope, the pathologist has a difficult time, in the words of a  
15 cliché, of seeing the forest for the trees. In the present invention, the pathologist has a full, magnified, reduced in size specimen view with the higher image area marked thereon to guide him and to let him see the forest. He can also see a region of the forest at higher  
20 magnification by scrolling adjacent tree images onto the high magnification screen.

In accordance with a further aspect of the invention, the user may elect to view an intermediate magnification and resolution. In the preferred system,  
25 at least three magnification images are acquired and stored as digitized images. The intermediate resolution images may be acquired by either switching automatically to a new objective lens and acquiring new digitized image tiles at the intermediate magnification or by using  
30 software to reconstruct a new intermediate digitized image from the existing high and low magnification, digitized images.

The preferred, low magnification image, which is reduced in size, can be transmitted over narrow band  
35 width channels such as a local area network or over the Internet through various servers and computers.

-13-

Likewise, the reconstructed, high magnification images can be transmitted over such narrow band width channels.

The preferred microscope is fully computer controlled, a pathologist or other person having a split  
5 screen computer such as a PC, can be connected to the microscope and operate it from a remote location with the computer microscope system to digitize and construct the macro image. Likewise, the pathologist can navigate to  
10 points of interest and with the computer microscope system to digitize and construct the desired micro images. With the present invention, there is no need for a specialized microscope at each remote location nor for a broad band channel to send video signals in real time between the diagnostic center and the remote location.

15 **Brief Description of the Drawings**

FIG. 1 is a screen view of a system embodying the present invention showing a low magnification image of a specimen on a microscope slide in one window, a high magnification image of a portion of the low magnification  
20 image selected by a region marker and a control window;

FIG. 2 is a view of a display screen of the apparatus embodying the present invention showing the control window a low magnification window having a plurality of high magnification micro image regions  
25 delineated therein and a high magnification window including one or more of the micro image regions;

FIG. 3 is a view similar to FIG. 2 including the control window but also including a low magnification region from the slide showing regions marked by a  
30 histology grade or structure through automatic analysis of tissue and a high magnification window showing markings related to the grading or histology grade yielded by the automatic analysis of tissue in combination with a window showing a numerical score;

35 FIG. 4 is a block diagram of the apparatus embodying the present invention;

-14-

FIG. 5 is a block diagram of a portion of the apparatus shown in FIG. 4 showing details of a mechanical arrangement of a microscope;

FIG. 6 is a flow diagram related to operation of  
5 the apparatus;

FIG. 7 is a flow diagram of details of one of the steps in FIG. 6;

FIG. 8 is a display screen showing control parameters to be manipulated thereon;

10 FIG. 9 is a flow chart for a region outlying routine;

FIG. 10 is a flow chart for a scanning and analyzing routine;

15 FIG. 11 is a schematic showing of the limits of travel of the microscope stage with respect to the image tiles;

FIG. 11A is a perspective view of the microscope stage and stepper motors and encoders providing a closed loop drive for the motors;

20 FIG. 12 is a block diagram of a networked system allowing multiple workstations to obtain access to diagnostic image information and to manipulate such information locally at each workstation;

FIG. 12A is a view of the system described in  
25 connection with FIG. 5;

FIG. 13 is a block diagram of a remote networked system for distributing and accessing diagnostic images and data through a hypertext transport protocol based server directly or over a packet network;

30 FIG. 14 is a view of a low magnification, reconstructed image from a basal layer of rat esophagus;

FIG. 14A is a view of a high magnification, reconstructed image from a selected point of interest from FIG. 14;

35 FIG. 15 is a view of a low magnification image of a mouse colon having a basal layer;



-15-

FIG. 15A is a view of a reconstructed macro image of a mouse colon;

FIG. 16 is a schematic view of an analysis from regions of a basal layer;

5           FIG. 16A is a schematic view of an analysis to provide to a Z score; and

FIG. 17 is a schematic view showing texture analysis tests for regions.

#### Detailed Description of the Preferred Embodiment

10           Referring now to the drawings, and especially to Figs. 4 and 5, apparatus for synthesizing low magnification and high magnification microscopic images is shown therein and generally identified by reference numeral 10. The system includes a computer 12 which is a  
15 dual Pentium Pro personal computer in combination with a Hitachi HV-C20 video camera 14 associated with a Zeiss Axioplan 2 microscope 16. The computer system 12 is able to receive signals from the camera 14 which captures light from the microscope 16 having a microscope slide 18  
20 positioned on an LUDL encoded motorized stage 20. The encoded motorized stage 20 includes a MAC 2000 stage controller for controlling the stage in response to the computer 12. A microscope slide 18 includes a biological specimen 21 which is to be viewed by the microscope and  
25 whose image is to be digitized both at low magnification and at high magnification as selected by a user. The low magnification digitized image is then displayed on a 21 inch Iiyama video display monitor 22 having resolution of 1600 by 1200 to provide display screens of the type shown  
30 in Figs. 1 through 3 including a low magnification image 24, for instance, at 1.25 power, a high magnification image 26, for instance at 40 power and a control window or image 28. The low magnification image may have  
35 identified therein a region 30 which is reproduced at high magnification in high magnification screen or window 26 so that a pathologist or other operator of the system

-16-

can review architectural regions of interest in low magnification image 24 and simultaneously view them in high magnification in the high magnification screen or window 26 to determine whether the cells forming a  
5 portion of the architectural feature need be examined further for cancer or the like or not.

The computer 10 is constructed around a PCI system bus 40 and has a first Pentium Pro microprocessor 42 and a second pentium pro microprocessor 44 connected  
10 thereto. The system bus 40 has connected to it a PCI bus 50 and an ISA bus 52. The PCI bus 50 has a SCSI controller 60 connected thereto to send and receive information from a hard disk 62. The hard disk 62 also is coupled in daisy chain SCSI fashion to a high capacity  
15 removal disk and to a CD Rom drive 66. The hard disks 62 contains the programs for operating the system for controlling the microscope 16 and for processing the images as well as for doing a quantitative analysis of the selected portions of the histological specimens being  
20 viewed on the slide 18. The system bus 40 also has connected to it a random access memory 70 within which portions of the program being executed are stored as well as a read only memory 72 for holding a bootstrap loader as well as portions of the basic input/output operating  
25 system. A floppy disk controller 74 is coupled to the system bus 40 and has connected to it a floppy disk drive 76 for reading and writing information to a floppy disk as appropriate. A mouse controller 80 is coupled to the system bus and has a mouse 82 which operates as a  
30 pointing device for controlling manipulations on the screen 22 and within the windows 24, 26 and 28. A keyboard controller 90 is connected to the system bus and has a keyboard 92 connected thereto. The keyboard 92 may be used to send and receive alpha numeric signals to  
35 other portions of the computer. An audio controller 100 has a plurality of speakers 102 and a microphone 104 connected thereto for audio input and output and is

-17-

coupled to the system bus 40. A network interface, such as a network interface card 104, is connected to the system bus and can provide signals via a channel 106 to other portions of a network or internet to which the system may be connected. Likewise, signals can be sent out of the system through a modem 110 connected to the ISA bus 52 and may be sent via a channel 112, for instance, to the internet. A printer 116 is connected via a parallel I/O controller 118 to the system bus in order to provide printouts as appropriate of screens and other information as it is generated. A serial I/O controller 122 is connected to the system bus and has connected to it a camera controller 124 which is coupled to CCD sensors 126 in the cameras. The CCD sensors 126 supply pixel or image signals representative of what is found on the slide 18 to an Epix pixci image acquisition controller 130 coupled to the PCI bus 50.

The microscope 16 includes a base 140 having a stage 20 positioned thereon as well as an objective turret 142 having a plurality of objectives 144, 146 and 148 thereon. The objective 144, for instance, may be of 1.25x objective. The objective 146 may be a 20X objective. The objective 148 may be a 40X objective. Signals from the camera sensors and controller are supplied over a bus 128 to the image acquisition system where they are digitized and supplied to the PCI bus for storage in RAM or for backing storage on the hard disk 62.

When a specimen is on the slide 18 the stage 20 may be manipulated under the control of the computer through a stage controller 160 coupled to the serial I/O controller 122. Likewise, a microscope controller 162 controls aspects of the microscope such as the illumination, the color temperature or spectral output of a lamp 168 and the like. For instance, in normal operation, when a specimen is placed on the slide, specimen slide 18 is placed on the stage 20 in a step

-18-

200, as shown in FIG. 6, the processors 42 or 44 send a command through the system bus to cause the serial I/O controller 122 to signal the microscope controller to change magnification to 1.25X in a step 202. This is  
5 done by rotating the objective turret of the Axioplan 2 microscope to select the objective 144. Likewise, the controller sets the color temperature of the lamp 168, sets a pair of neutral density filter wheels 170 and 172 and sets a field diaphragm 174 for the correct  
10 illumination. A condenser diaphragm 176 is also controlled and a color filter wheel 180 may also be controlled to apply the appropriate filter color to the CCD sensors 126 in the camera. The entire slide is then scanned in a step 204. The images are tiled and melded  
15 together into the overall image 24 supplied on the screen 22 to provide the operator in the step 206 with a visually inspectable macro image of relevant regions of the slide of interest.

In order to provide the magnified image, the  
20 mouse may be moved to identify a marker segment or region which, for instance, may be a rectangular region (as shown as 30 in FIG. 1) which will cause the microscope to change magnification as at step 208 to 4x, 20x, 40x, etc., by rotating the turret to bring the appropriate  
25 objective lens system into viewing position.

Next the user, in a step 209a, uses the mouse to select the region on the macro image in order to select the micro image to be viewed on the screen 22. In a step 209b a test is made to determine whether the user has  
30 commanded continued inspection. If the user has, a test is made in a step 209c to determine if the magnification is to be changed by changing the selected objective. In the event the magnification is to be changed control is transferred to the step 208. If the magnification is to  
35 remain unchanged control is transferred to the step 209a. In the event inspection is not to continue the region selected is outlined for higher magnification scan in a

-19-

step 209d. In a step 209e, a command may be received to scan or acquire the higher magnification image for display in screen 26. The image may then be archived for later analysis, displayed or analyzed immediately.

5           In order to perform the magnification called for in step 208, the overall illumination and control of the microscope will be controlled so that in a step 210 the objective turret 142 will be rotated to place the higher power objective above the slide 18. In a step 212  
10 voltage to the lamp will be changed to adjust the lamp 168 to provide the proper illumination and color temperature as predetermined for the selected objective. In a step 214, the condenser diaphragm 176 will have its opening selected as appropriate to provide the proper  
15 illumination for that objective. In a step 216, the filter turret 180 will select the proper light wavelength filter to be supplied to the camera sensors. For instance, a red, blue or green filter, as appropriate, particularly if the specimen has been stained. In a step  
20 218 the field diaphragm 174 will have its opening changed. In a step 220 the neutral density filter wheel 170 will select a neutral density filter and in a step 222 the neutral density filter wheel 172 will also select a neutral density filter. In a step 224 the X, Y and Z  
25 offsets will be used for reconstruction of the recorded image at the magnification and in a step 226 the current position will be read from encoders in the stage which are accurate to .10 micron.

          In order to identify the selected region the  
30 mouse is moved to that area of the region in a pointing operation in a step 240 as shown in FIG. 9. The mouse may be moved to draw a box around the region selected. In a step 242 the X and Y screen points are computed for the edges of the regions selected and the computed image  
35 or pixel points are translated to stage coordinate points in order to control the stage of the microscope. In a step 244 a list of all of the X fields for positioning

-20-

the stage for the objective is stored in random access memory and may be backed up on the hard disk. The information from the X offsets for the objective and the stage offsets is used as well as the size of the field to position the slide properly under the objective to capture the micro image.

When the slide has been positioned properly, as shown in FIG. 10 in a step 250 the stage is positioned for each of the X and Y coordinate values in stage coordinate values and the digitized image is captured by the cameras and stored in RAM and backed up on the hard disk. The image may be then analyzed quantitatively in various manners such as those set forth in the previously-identified United States application. Optionally the image may be stored for archival purposes in a step 254.

In order to override the specific control functions that take place as shown in FIG. 7, a screen is provided as shown in FIG. 8 wherein the XY step size can be edited, the X, Y and Z offset can be edited, the lamp voltage can be selected, the neutral density filter can be selected as well as the opening of the field diaphragm and several other microscopic characteristics. FIG. 8 is a view of the settings of the microscope objective properties of the Axioplan 2, computer-controlled microscope.

The X and Y positioning is specifically carried out as shown in FIG. 11 where the slide 18 is shown with a slide boundary 270, 272, 274 and 276. Stage boundary for limits of the stage travel for purposes of the stage the stage can be moved all the way from an upper left hand corner of travel 276 to a lower right hand corner of travel 280. At the upper left hand bounded corner of travel 278 limits which a signal that the end of travel has been reached and the stage is then translated a short distance 282 in the extra action and a short distance 284 in the Y direction to define the first tile 288 in terms

-21-

of a reference point 290 at its upper left hand corner. Since the size of the macro image tile 288 is known, the next macro image tile 292 may be placed contiguous with it by moving the stage appropriately and by measuring the  
5 location of the stage from the stage in counters without the necessity of performing any image manipulation. The image tiles 288 and 292 may be abutted without any substantial overlap or they may be overlapped slightly, such as a one pixel with overlap, which is negligible  
10 insofar as blurring of any adjacent edges of abutted image tiles. The upper left hand corner 300 of the tile 292 defines the rest of 292 and other tiles can be so defined. Micro image tiles can likewise be defined so that they are contiguous but not substantially  
15 overlapping, as would interfere with the composite image. This avoids the problems encountered with having to perform extended computations on digital images in a frame storer or multiple frame storage in order to match or bring the images into contiguity without blurriness at  
20 the edges of contiguous image tiles. It may be appreciated as shown in FIG. 2 that the low power image 24 has a plurality of micro images defined therein which are tiled and which are shown in higher magnification as individual tiles 312, 314, 316 and the like in FIG. 2.  
25 In addition, the region 310 when magnified as shown in the window 26 may exceed the bounds of the window and thus the window may include scroll bars or other means for allowing the image 310 which is larger than the window 26 to be examined from within the window 26.

30 The stage 200 is best seen in FIG. 11A and includes the X and Y stepper motors 279 and 281 with their respective encoders, which provide a closed loop system to give the .1 micron accuracy versus the usual 5 or 6 micron accuracy of most microscope stages without a  
35 closed loop system. This closed loop system and this very high accuracy allow the abutting of the tile images for both high magnification and low magnification images

-22-

without the substantial overlap and the time-consuming and expensive software currently used to eliminate the overlap and blurriness at the overlapping edges of adjacent image tiles. With the precisely positioned stage and by using the tiling system described in connection with FIG. 11, where the slide is precisely positioned relative to a center point CP for the slide, and the known position of point 278 is always taken from the same point, the tiles may be positioned precisely in a horizontal row and precisely in vertical rows to reconstruct the macro image and the micro image. This reconstruction is done without the use, as in the prior art, of extensive software manipulation to eliminate overlapping image tiles, horizontally or vertically or the haphazard orientation of image tiles.

Furthermore, as shown in FIG. 3, the low power window 24, high power window 26 and control window 28 can be used in conjunction with reporting of quantitative analysis data, histograms, etc. for the specimen being viewed; and such analysis information may be provided as a visual output in a window 320. Each of the various regions 30 that a pathologist may follow in marking various features in the low power window 24 and the high power window 26 may be reflected in both windows in order that an audit trail is provided for the system.

The present invention also includes the facility for allowing remote diagnostics to occur by being able to couple the system either over a network communication facility to an intranet, for instance via the network interface, or via a modem or other suitable connection, to an internet so that once the image has been scanned and stored in memory on hard disks or other storage, remote users may be able to access the low magnification image as well as the high magnification image and move around within both images to make determinations as to the histological characteristics of the samples via Z scores.



-23-

An additional feature of the system includes a plurality of networked workstations coupled to a first computer console 12 having a display screen 22 connected to the microscope 14. Satellite work stations 350 and 5 352 are substantially identical to the work station 12 including respective computers 354 and 356 coupled to displays 358 and 360. The devices can be manipulated through input devices 360 and 362 which may include a keyboard, mouse and the like. Also a third device can be 10 connected including a work station 370, having a display 372, a computer 374 and an input device 376. Each of the devices is connected over respective network lines 380, 382, 384 to the computer 12 which transmission may be via either net or the like. Each of the different operators 15 at the physically separate viewing stations can locate regions from the view of entire tissue cross sections via a macro view and label the regions for subsequent scanning and/or quantitative analysis. A single operator at the instrument station 12 can locate regions to view 20 the entire tissue cross section. Those regions can be labeled for subsequent scanning and/or quantitative analysis with subsequent review and physically remote viewing stations, for instance, in an operating room or in individual pathologists' signout areas in order to 25 review analysis results while still maintaining and reviewing the entire macro view of the tissue and/or the individual stored images from which the quantitative results were obtained. The viewing stations 350, 352 and 370 can comprise desk top computers, laptops, etc. There 30 is no need for a microscope at the network stations 350, 352 and 370.

In a still further alternative embodiment, remote workstations 400, 402, 404, 406 and 408 may be connected through a server 410 which may be supplied via 35 a packet switched network. The server 410 and may be a hypertext transport protocol based server of the type used for the World Wide Web or may be a telnet type

-24-

server as used previously in internet remote operation applications. The server 410 communicates via a communications channel 414 with a local computer 416 having a display 418 associated therewith, the local  
5 computer 416 being connected to the microscope 420. Each of the remote work stations 400, 402, 404, 406 and 408 may perform the same operations as the stations 350, 352 and 370 although they do it from nearby buildings or even from around the world, thus providing additional  
10 flexibility for others to make use of the specimen obtained and being viewed under the microscope 420. In addition, stored images may be disseminated through the server 410 to the remote servers 400 through 408 for further analysis and review.

15 In FIG. 14, there is illustrated on screen 28 a basal layer 431a of a cut cross-section of a rat esophagus. The basal layer is elongated and linear in a downward direction, and the selected point of interest is shown as a box 30 on the basal layer on the composite,  
20 low magnification image. The high magnification image 26 of this selected point of interest is shown on screen 26 in FIG. 14A. In FIG. 15 is shown a mouse colon as a reconstructed, low magnification macro image 28 which has been reduced 1/16th in size. The micro image 26 is shown  
25 in FIG. 15A, and the marking therefore is shown in FIG. 15.

The analysis for texture and for morphological features used to analyze a series of regions 30 on the elongated basal layer that were analyzed at high  
30 magnification are shown in FIGS. 16, 16A and 17. The manner of doing these tests and of obtaining a Z score or grade is disclosed in the aforesaid patent application.

While there has been illustrated and described a particular embodiment of the present invention, it will

-25-

be appreciated that numerous changes and modifications  
will occur to those skilled in the art, and it is  
intended in the appended claims to cover all those  
changes and modifications which followed in the true  
5 spirit and scope of the present invention.

-26-

**What Is Claimed Is:**

1. A method of using a computer-controlled microscope to acquire and store multiple resolution images from a specimen on a microscope support, said  
5 method comprising the steps of:

digitally scanning and storing a first set of digitized images taken from a portion of a specimen on a microscope slide in a first set of contiguous image tiles at a first magnification to allow formation of an overall  
10 view at a first magnification resolution;

digitally scanning and storing a second set of digitized images taken from the portion of the specimen on the microscope slide in a second set of contiguous image tiles at a second, higher magnification; and

15 addressing the first and second digitized images so that the higher magnification images can be easily located from the lower magnification images.

2. The method of Claim 1 wherein the addressing step further comprises providing each of the image tiles  
20 in the first series and in the second series with coordinate information for enabling reconstruction of the first and second images.

3. The method of Claim 1 wherein each of the digitized images of the series corresponds to  
25 substantially an optical view as seen by a person through an objective lens of the microscope.

4. The method of Claim 1 including the step of remotely operating the controlled microscope to select a segment of the specimen from the first magnification view  
30 for digitally scanning and storing the second set of images tiles.

5. A method of using a computer-controlled microscope imaging system for acquiring and storing

-27-

digitized microscopic images from a specimen on a microscope support, said method comprising the steps of:

scanning and digitizing a specimen at a low magnification through a microscope to acquire digitized  
5 images;

displaying to the observer a low magnification, digitized image of the specimen;

selecting a segment of the specimen from the low magnification digitized image for viewing at a higher  
10 magnification greater than the low magnification;

scanning the selected segment at the higher magnification and digitizing the image of the segment to acquire digitized images; and

storing the respective, low magnification, digitized images and the high magnification, digitized  
15 images of the segment.

6. The method of Claim 5 wherein the step of scanning and digitizing at low magnification further includes the steps of:

20 positioning and coordinating spatially a plurality of low magnification image tiles spatially to provide a composite macro image of the specimen formed from the plurality of low magnification image tiles; and

providing a macro image of the specimens having  
25 a field of view substantially larger than the field of view yielded by the objective lens used for the low magnification scanning.

7. A method in accordance with Claim 6 including the step of reducing the size of the composite, macro image before displaying the composite macro image  
30 of the specimen to the viewer.

8. A method in accordance with Claim 6 wherein the step of selecting and digitizing the low magnification, digitized image further includes the step

-28-

of positioning and coordinating spatially a plurality of low magnification images spatially to provide a composite image having a field of view substantially larger than the field of view yielded by the objective lens used for  
5 high magnification scanning.

9. A method in accordance with Claim 5 in which the step of the selecting a defined segment area of interest further includes the step of moving a marker on the low magnification image to a point of interest to be  
10 viewed at high magnification; and operating the microscope to change objectives and conditions of illumination to provide a good image at high magnification.

10. A method in accordance with Claim 9 wherein  
15 the high and low magnification images are each reconstructed from image tiles each having a spatially known location used to assure their proper positioning in the reconstructed image.

11. A method in accordance with Claim 10  
20 wherein the step of automatically changing from one magnification scanning operation to another magnification scanning operation which includes one or more of the steps of:

changing the microscope objective lens to a  
25 different magnification;  
changing at least one filter;  
changing the field diaphragm;  
changing the light intensity;  
changing the focus; and  
30 shifting the microscopic stage.

12. The method of Claim 9 including the step of displaying the low magnification image of the specimen and displaying the marker on the low magnification image,

-29-

and simultaneously displaying the high magnification image of the segment at a position located by the marker on the low magnification image.

13. The method of Claim 5 including the step of  
5 shifting the marker to different segments and viewing at high magnification each of the selected segments; and marking on the low magnification image, each of the segments viewed at high magnification.

14. The method of Claim 5 including the steps  
10 of locating a computer-controlled microscope system used to make and store the digitized images on the Internet having a web site therefor; including the step of connecting an observer's computer and viewing screen over the Internet to the computer-controlled microscope at the  
15 web site in real time and transmitting the digitized signals for low magnification image and high magnification image from the web site to the observer's computer and viewing screen.

15. The method of Claim 5 including the step of  
20 enhancing the reconstructed, high magnification image by color filtering.

16. The method of Claim 5 including the step of providing a full color, low magnification, digitized image to the observer for aiding the observer in  
25 selecting a plurality of areas of interest on a priority basis.

17. The method of Claim 5 including the steps  
of:  
analyzing the specimen on the slide with image  
30 analysis tests; and

-30-

reporting the results of the tests to the observer while at least one of the respective images is being viewed by the observer.

18. The method of Claim 17 wherein the specimen  
5 is a biological specimen, and the analyzing step including performing analysis tests on tissue or cells in the biological specimen.

19. A method in accordance with Claim 5  
including the step of the observer changing the  
10 magnification of the high magnification image to a magnification intermediate the high and low magnification images by the use of an image analysis algorithm.

20. A method in accordance with Claim 5  
including the step of and scrolling the high  
15 magnification image tiles to bring into view on a screen adjacent sections not previously viewed.

21. A method of using a computer-controlled microscope imaging system for acquiring and analyzing areas of interest, said method comprising the steps of:  
20 scanning and digitizing a specimen at a low magnification through a microscope;  
displaying to the observer a low magnification, digitized image of the specimen;  
selecting a segment of the specimen from the low  
25 magnification digitized image for viewing at a higher magnification greater than the low magnification;  
automatically scanning the selected segment at the higher magnification and digitizing the image of the segment; and  
30 making available in real time to the view of the observer the respective, low magnification, digitized image and the high magnification, digitized image of the segment.



-31-

22. The method of Claim 21 wherein the step of scanning and digitizing at low magnification further includes the steps of:

positioning and coordinating spatially a  
5 plurality of low magnification image tiles spatially to provide a composite macro image of the specimen formed from the plurality of low magnification image tiles; and  
providing a macro image of the specimens having  
a field of view substantially larger than the field of  
10 view yielded by the objective lens used for the low magnification scanning.

23. A method of using digitized, microscope images of a specimen having images at a first resolution at a low magnification and images at a second resolution  
15 at a higher magnification, the method comprising the steps of:

showing to a user a series of digitized, tiled and stored images abutted to form a macro image from the specimen at a first resolution;  
20 selecting from the stored macro image an area thereon to the viewer to be viewed at higher resolution;  
showing to the user a series of abutted, digitized, tiled, stored images at the higher magnification and higher resolution to the viewer to be  
25 viewed at higher resolution.

24. A method in accordance with Claim 23 including the further step of shifting by user, back and forth between lower and higher resolution, stored images to analyze the specimen.

30 25. A method in accordance with Claim 24 including the further step of marking on the stored macro image the area to be viewed at higher resolution.

-32-

26. A method in accordance with Claim 23 including the step of scrolling to neighboring, tiled images for viewing areas on the specimen adjacent the selected area.

- 5           27. A method of acquiring and analyzing biological specimens with a computer-controlled microscope, said method comprising the steps of:
- scanning and digitizing a series of fields of views through a microscope objective lens of a low  
10 magnification and providing a series of digitized fields of view images;
- arranging and positioning the adjacent fields of view images and forming therefrom a composite, macro image in color of the specimen having a field of view  
15 larger than that of the objective lens;
- displaying the macro color image to the observer to provide the observer with a larger field of view of the specimen than the observer could see through an objective lens on the microscope;
- 20           selecting a segment of the specimen from the macro image for viewing at a higher magnification than the low magnification;
- scanning and digitizing a series of fields of view through a microscope objective lens of a higher  
25 magnification and providing a series of digitized images;
- arranging and positioning the adjacent fields of view images at high magnification and forming therefrom a composite micro image of the segment having a field of view larger than that of the high magnification objective  
30 lens; and
- displaying to the observer both colored composite macro image and the micro image each having a field of view substantially larger than their respective objective lens.

-33-

28. A method in accordance with Claim 27 including the step of marking on the macro image, the location of the micro image, so that the observer understands where the micro image is located on the macro  
5 image.

29. A method in accordance with Claim 27 including the step of selecting several segments each for viewing as a micro image and marking the location of each micro image on the composite image.

10 30. A method in accordance with Claim 29 including the step of recording the macro image with the marks thereon to provide a record of the segments viewed by the observer at high magnification to provide an audit trail for a later auditing of the segments viewed at  
15 higher resolution.

31. A method of acquiring and analyzing biological specimens with a computer-controlled microscope; said method comprising the steps of:  
providing a biological specimen in position for  
20 scanning through the computer-controlled microscope;  
scanning and digitizing the biological specimen through the microscope at a first, low magnification and acquiring and storing a digitized image of the specimen in color;  
25 displaying the stored, low magnification, digitized image in color to an observer to provide a macro image for viewing by the observer;  
selecting a segment of the colored, digitized, low magnification image for viewing at a higher  
30 magnification than the low magnification;  
scanning the segment at the higher magnification and acquiring a colored digitized image of the segment at the higher magnification to provide a micro image of the segment;

-34-

making available in real time to the view of the observer, the respective micro and the macro images; and performing a quantitative analysis of the specimen from the digitized high magnification image.

5           32. A method in accordance with Claim 31 including the step of marking on the macro image the position of the micro image so that the viewer understands the location of the micro view.

          33. A method in accordance with Claim 31  
10 including the steps of:  
          digitizing a plurality of fields of view through the microscope at each of the low and high magnifications to form a series of digitized image tiles;  
          arranging the respective image tiles to form the  
15 respective macro and micro images;  
          displaying to the observer the micro and macro digitized images that each have a larger field of view than a single field of view through respective objective lens for low and high magnification.

20           34. A microscopic system for analyzing a specimen and a segment of the specimen, said apparatus comprising:  
          a microscope having a plurality of objective lens to acquire images at different magnifications;  
25           a computer-controlled light illumination subsystem on the microscope for adjusting light illumination for different magnifications;  
          a computer-controlled, focusing subsystem on the microscope for adjusting the focus for different  
30 magnifications;  
          a computer-controlled X and Y stage to position the specimen to be viewed at specified X and Y coordinates;

-35-

an imaging subsystem connected to a microscope to acquire and digitize the images of the specimen;

a first optical system and image acquisition and image storing system having a first objective lens to  
5 acquire and arrange a stored series of images at a low magnification to provide a macro digitized image of the specimen having a field of view larger than the field of view from the objective lens used for the low magnification acquisition of images;

10 a second optical and image acquisition and image storing system having a second, higher magnification objective lens to acquire and arrange a stored series of images at higher magnification and to provide a micro view image of the segment having a field of view larger  
15 than the field of view from the higher magnification objective lens.

35. A system in accordance with Claim 34 wherein the first image subsystem includes an X and Y position storage device for storing the X and Y  
20 coordinates for the macro image.

36. A system in accordance with Claim 34 wherein the second image acquisition subsystem has a subsystem for reconstructing high magnification image tiles with their X and Y coordinates positioned to  
25 reproduce spatially high resolution image tiles into the micro image as if the micro image was in the original image.

37. A system in accordance with Claim 34 wherein the first image acquisition subsystem has a  
30 subsystem for reconstructing low resolution image tiles with their X and Y coordinates positioned to reproduce spatially the low resolution tiles into a macro image of the specimen.

-36-

38. A microscopic system for analyzing a specimen and a segment of the specimen, said apparatus comprising:

- 5 a microscope having a plurality of objective lens to acquire images at different magnifications;
- a computer-controlled light illumination subsystem on the microscope for adjusting light illumination for different magnifications;
- 10 a computer-controlled, focusing subsystem on the microscope for adjusting the focus for different magnifications;
- a computer-controlled X and Y stage to position the specimen to be viewed at specified X and Y coordinates;
- 15 an imaging subsystem connected to a microscope to acquire and digitize the images of the specimen;
- a first optical system and image acquisition system having a first objective lens to acquire and arrange a series of images at a low magnification to
- 20 provide a macro digitized image of the specimen having a field of view larger than the field of view from the objective lens used for the low magnification acquisition of images;
- a second optical and image acquisition system
- 25 having a second, higher magnification objective lens to acquire and arrange a series of images at higher magnification and to provide a micro view image of the segment having a field of view larger than the field of view from the higher magnification objective lens; and
- 30 a display screen device to make available in real time the micro and macro images on screens of the screen device.

39. A system in accordance with Claim 38 wherein the first image subsystem includes an X and Y

35 position storage device for storing the X and Y coordinates for the macro image.

-37-

40. A method for analyzing biological specimens by an image analysis system having a computer-controlled, automated microscope comprising the following steps:

- placing a biological specimen in a microscope
- 5 for viewing;
- using a computer terminal to control the image analysis system and microscope to acquire low magnification, image tiles of the specimen and to provide low magnification, composite, macro image from the
- 10 plurality of image tiles;
- displaying the macro image on a screen of the computer terminal;
- interactively selecting at least one point of interest on the displayed macro, specimen image for
- 15 viewing at a higher magnification;
- sending signals from the computer terminal to control the image analysis system and the computer-controlled microscope to acquire a plurality of higher magnification, image tiles and to provide a low
- 20 magnification, composite micro image; and
- analyzing the micro images of the specimen.

41. A storage medium having digitized images of a specimen from a microscope slide comprising:

- a storage medium;
- 25 a first series of contiguous, multiple digitized images at a first magnification abutted against each other to create an overall low resolution view of several adjacent, original microscope images assembled together;
- the first series of multiple, digitized images
- 30 taken from at least a portion of a specimen on the slide;
- a second series of contiguous, multiple, digitized images at a second higher magnification abutted against each other to create a high resolution view of several adjacent, original microscope images assembled
- 35 together, taken from said portion of the specimen; and

-38-

the first and second series of digitized images providing multiple resolution images of the slide specimen to a viewer.

42. A storage medium in accordance with Claim  
5 41 wherein each of the series of contiguous, multiple images corresponds to an optical image seen by a person through an objective lens of a microscope.

43. A storage medium in accordance with Claim  
41 wherein each of the series of contiguous, multiple  
10 images comprises reduced image of a corresponding original microscope image.

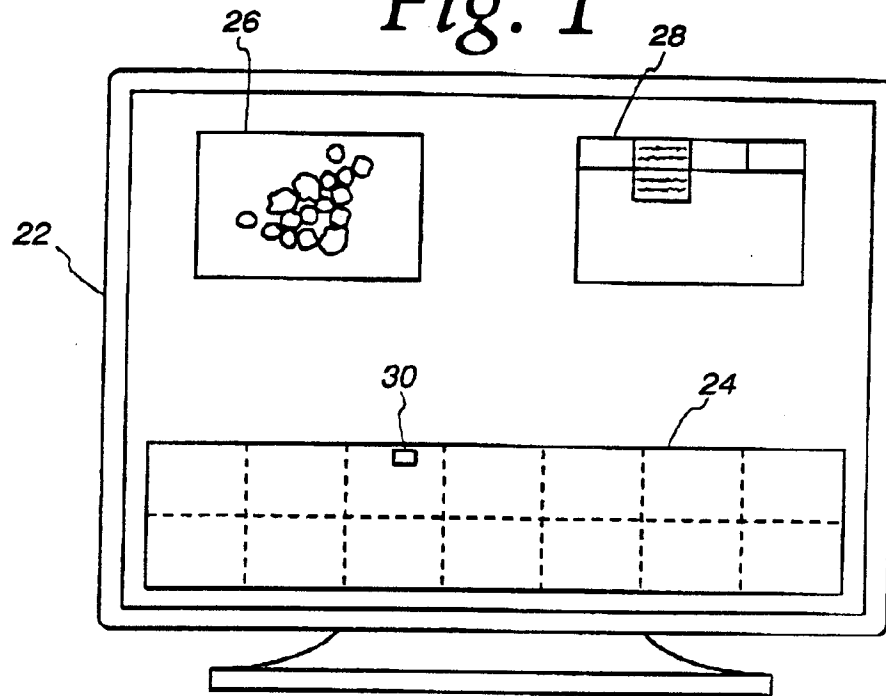
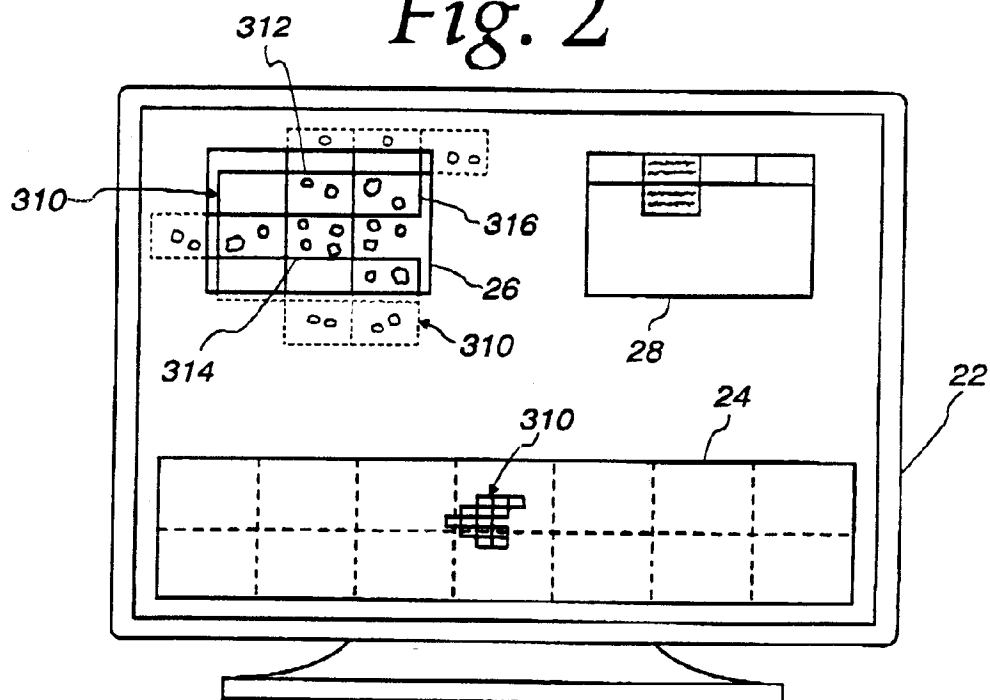
44. A storage medium in accordance with Claim  
41 wherein the storage medium comprises a RAM.

45. A storage medium in accordance with Claim  
15 41 wherein the storage medium comprises a hard disk.

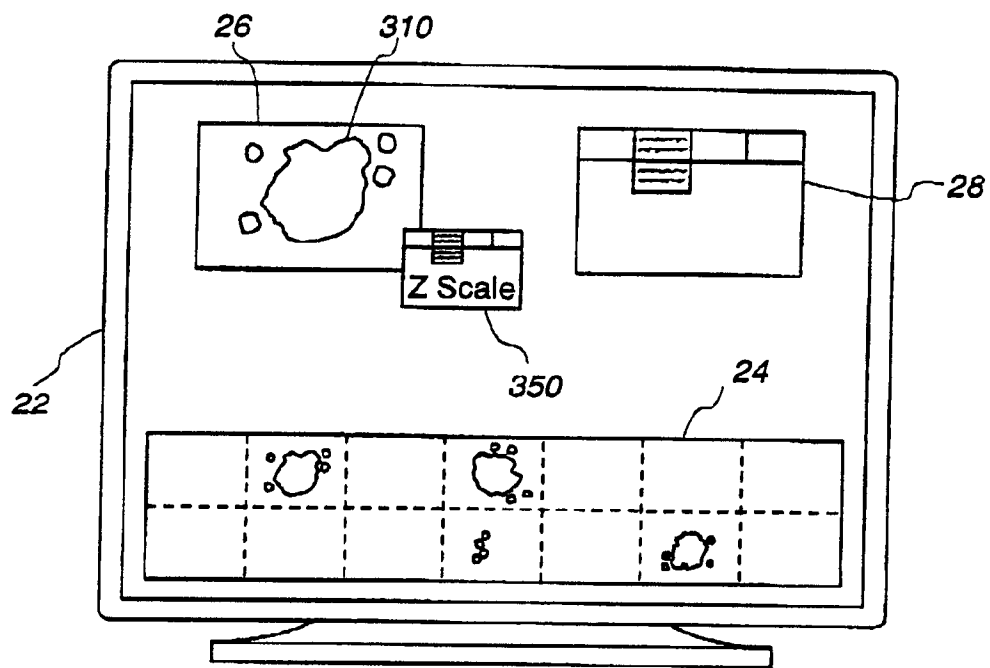
46. A storage medium in accordance with Claim  
41 wherein the stored images may be disseminated through a server to remote devices located on a communication channel.



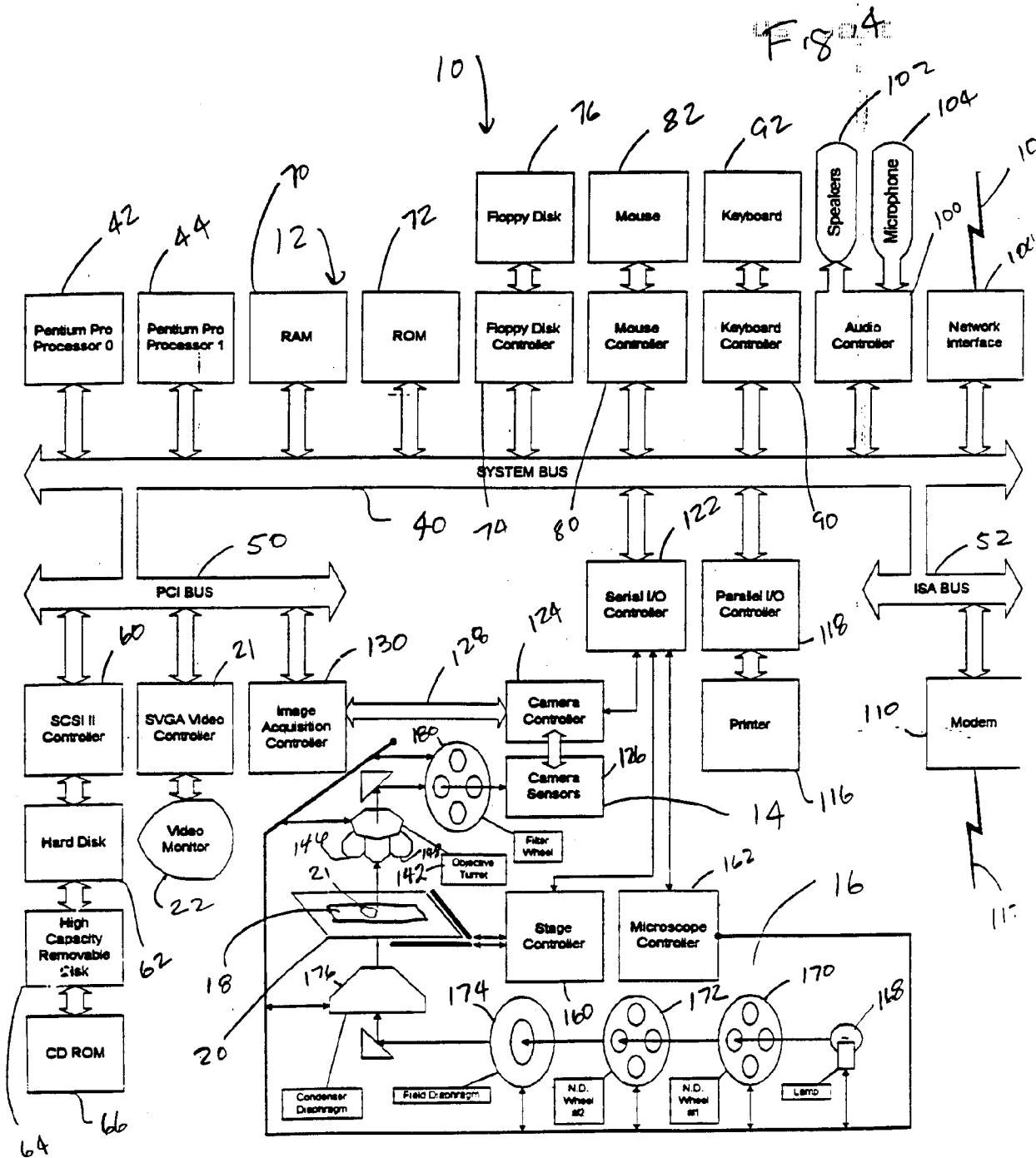
1/15

*Fig. 1**Fig. 2*

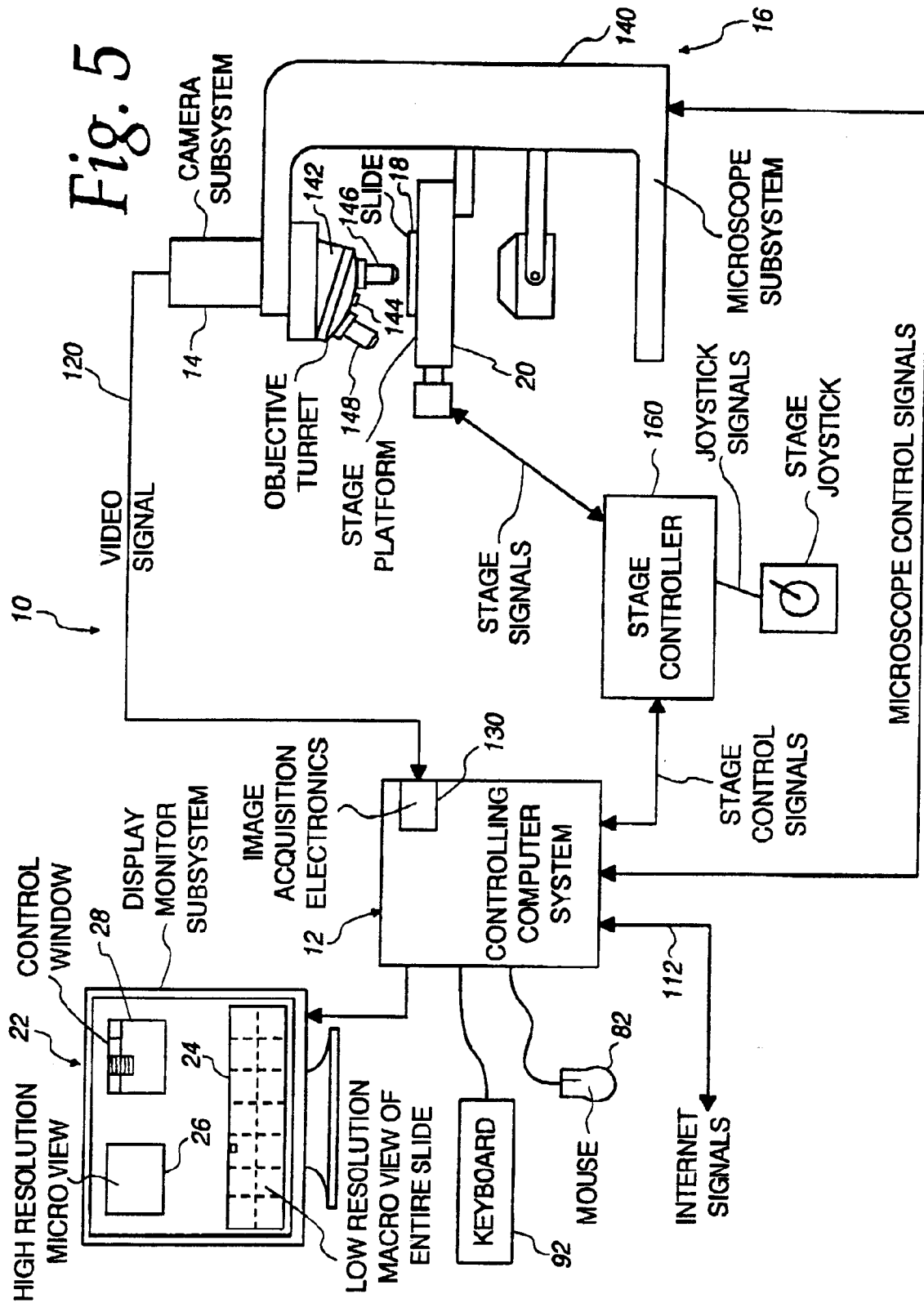
2/15

*Fig. 3*

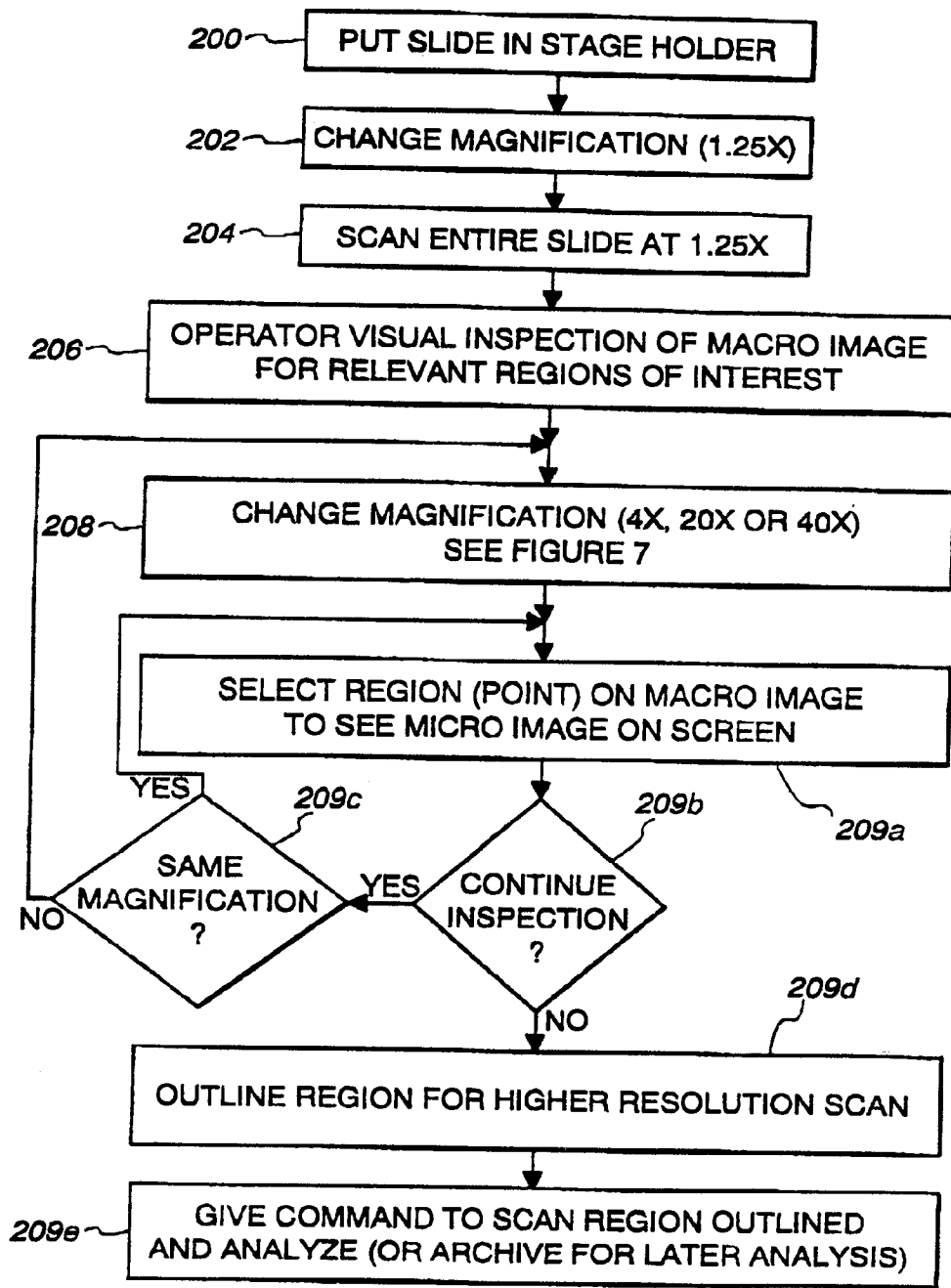
3/15



4/15

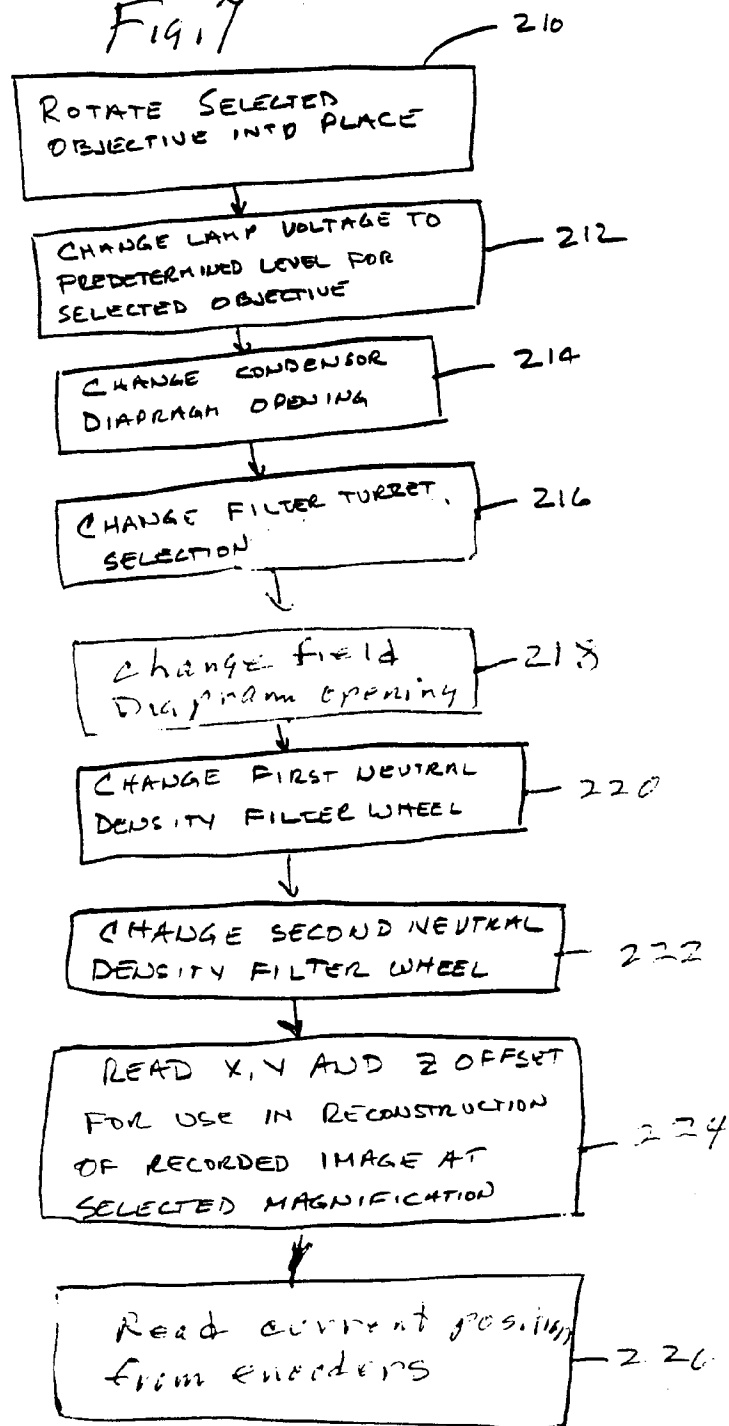


5/15

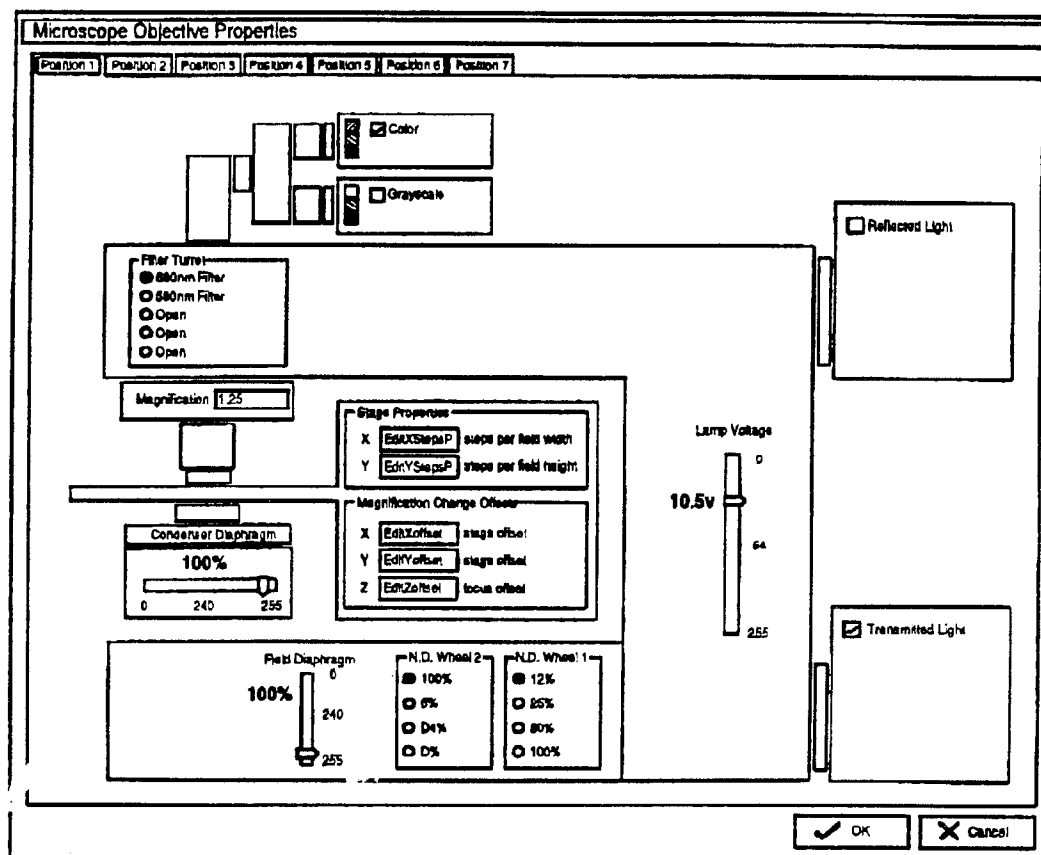
*Fig. 6*

6/15

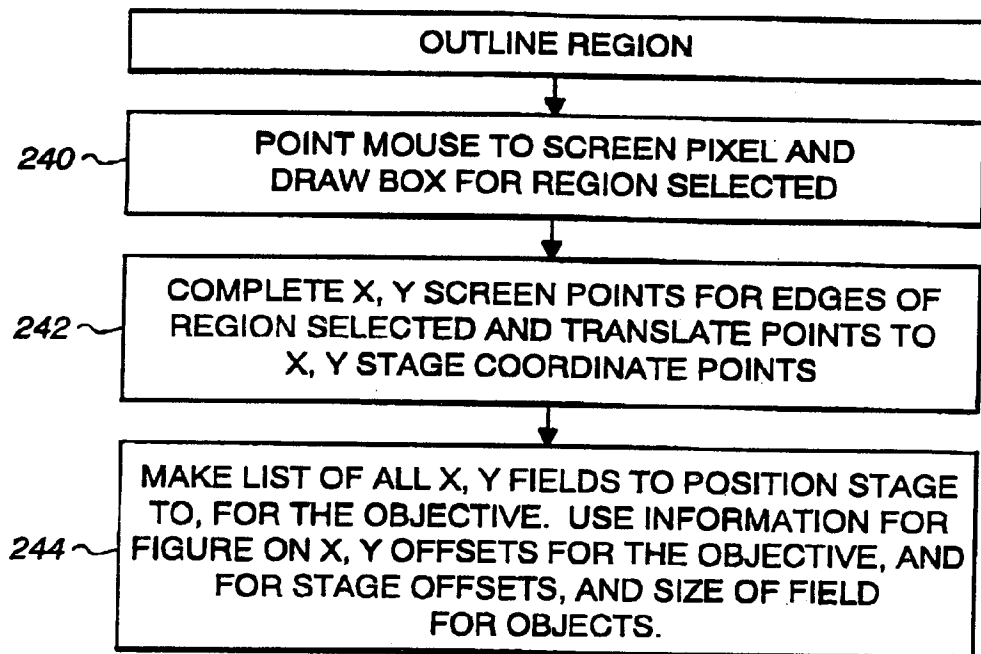
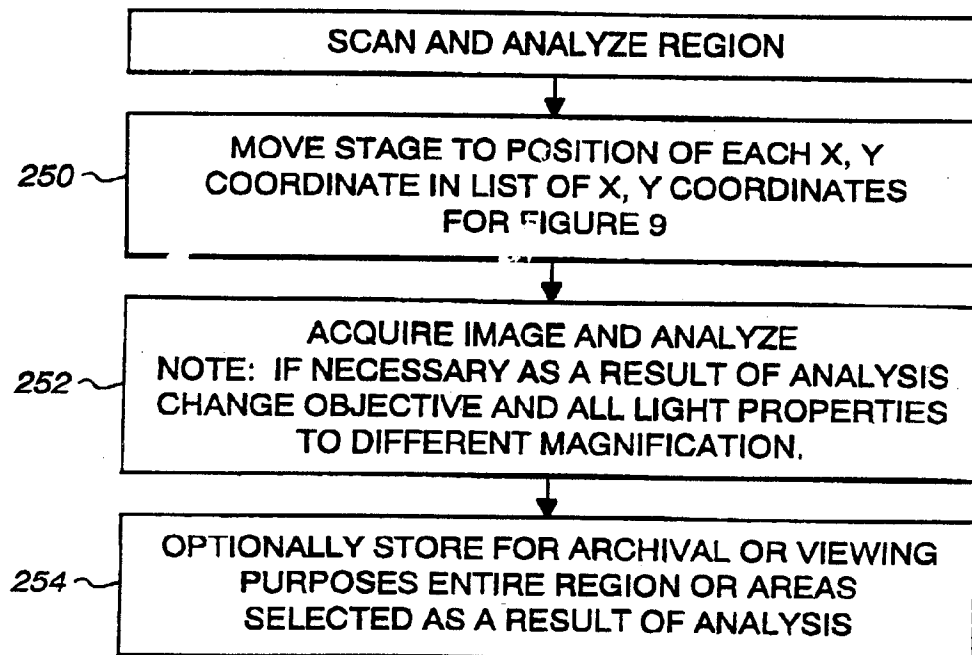
Fig. 7



7/15

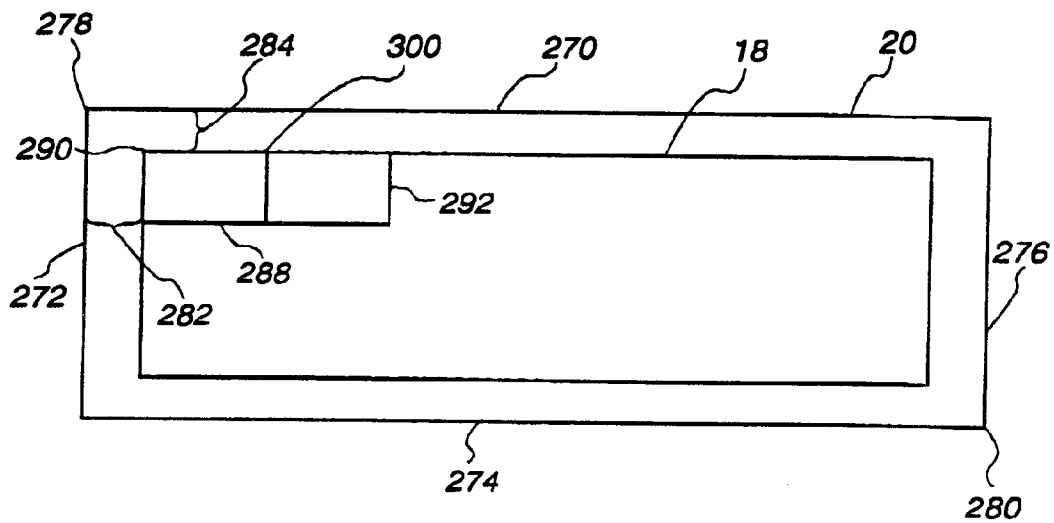
*Fig. 8*

8/15

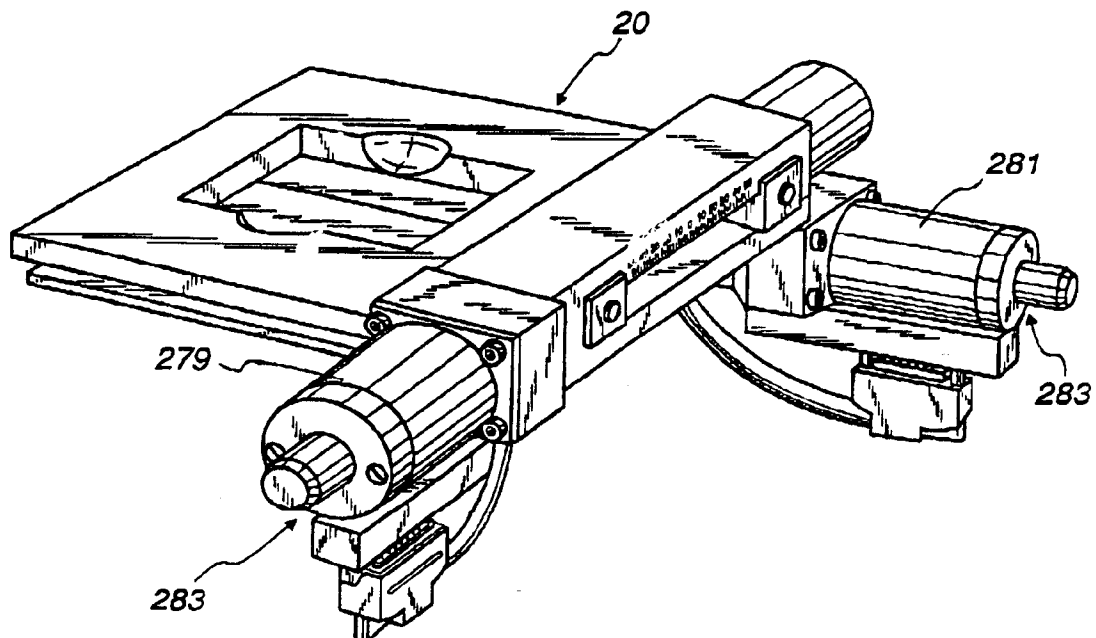
*Fig. 9**Fig. 10*



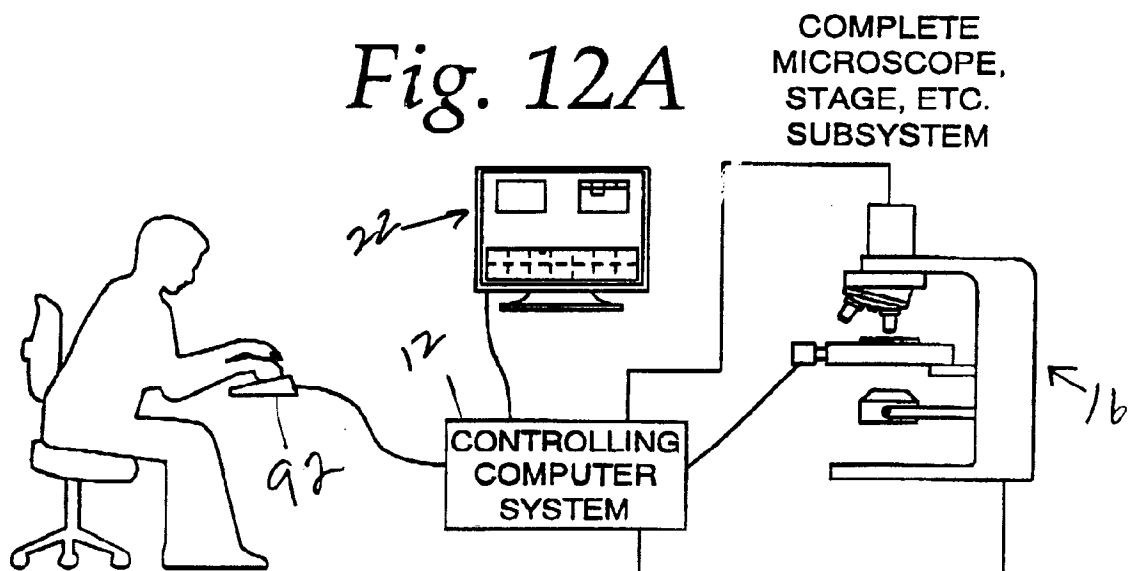
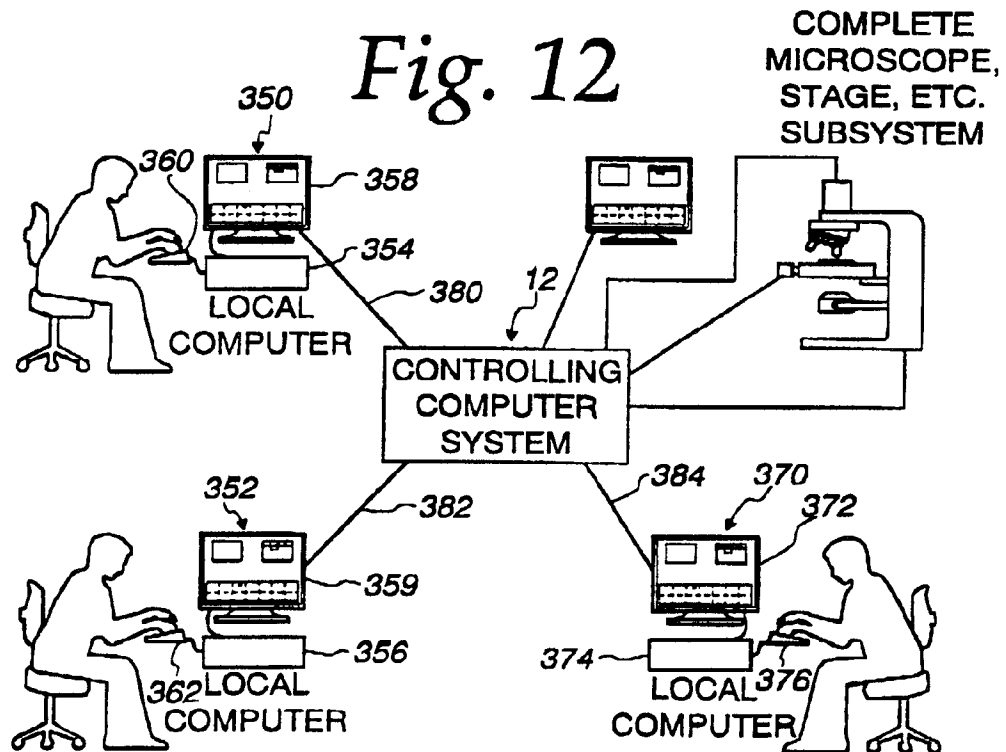
*Fig. 11*



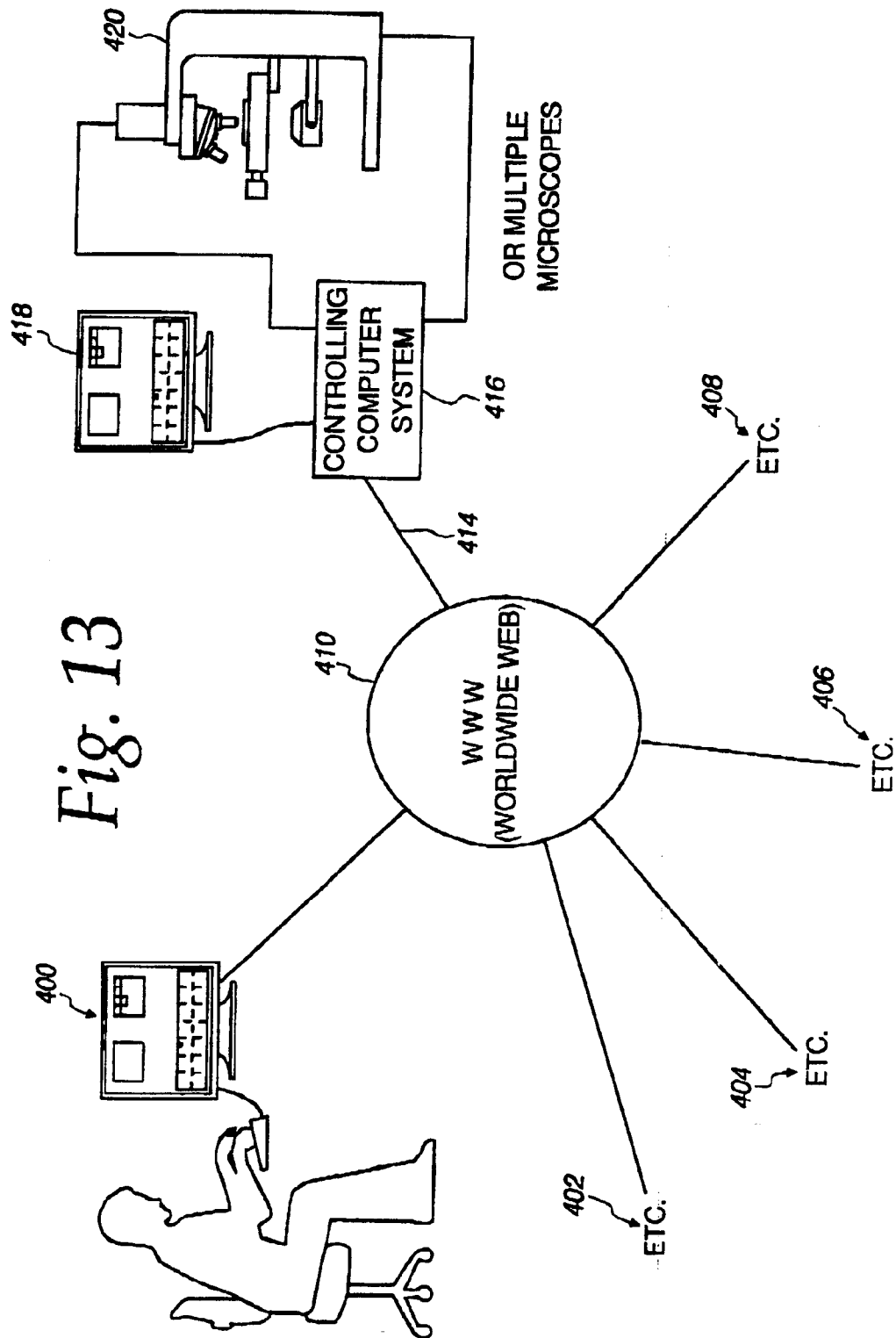
*Fig. 11A*



10/15



11/15



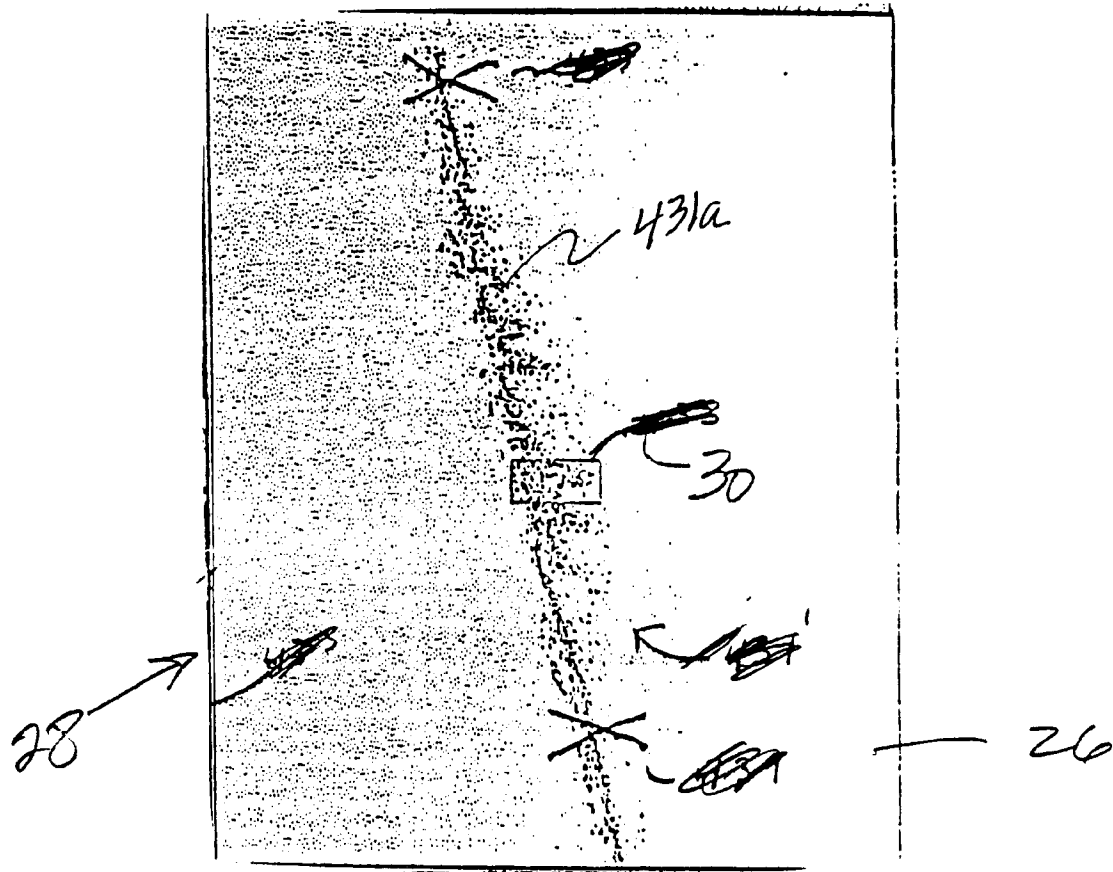


Fig. 14

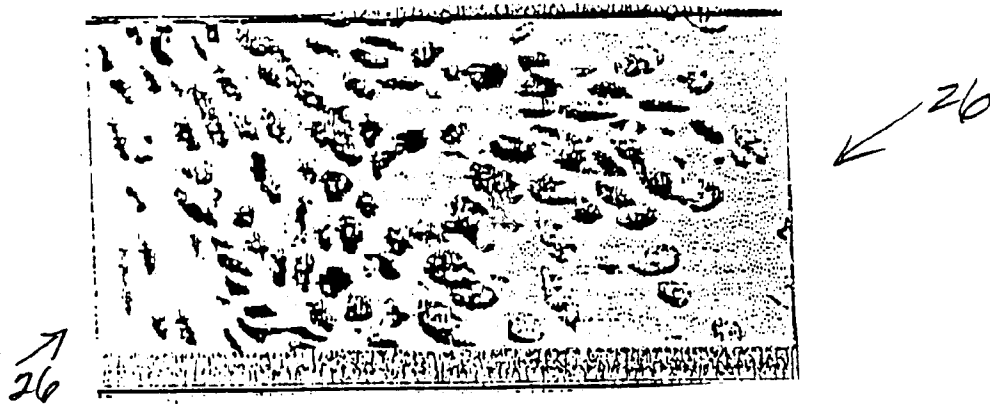
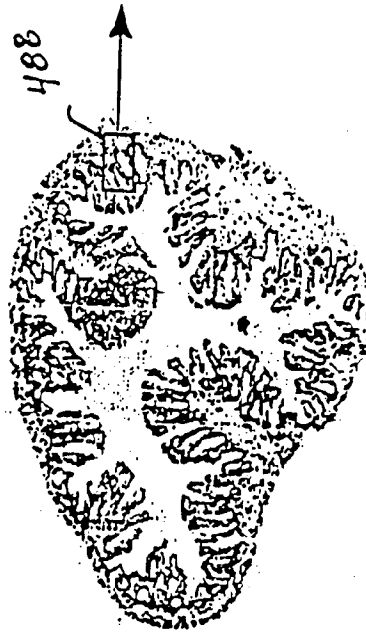


Fig. 14A

# Tissue Section Image Acquisition (Mouse Colon)

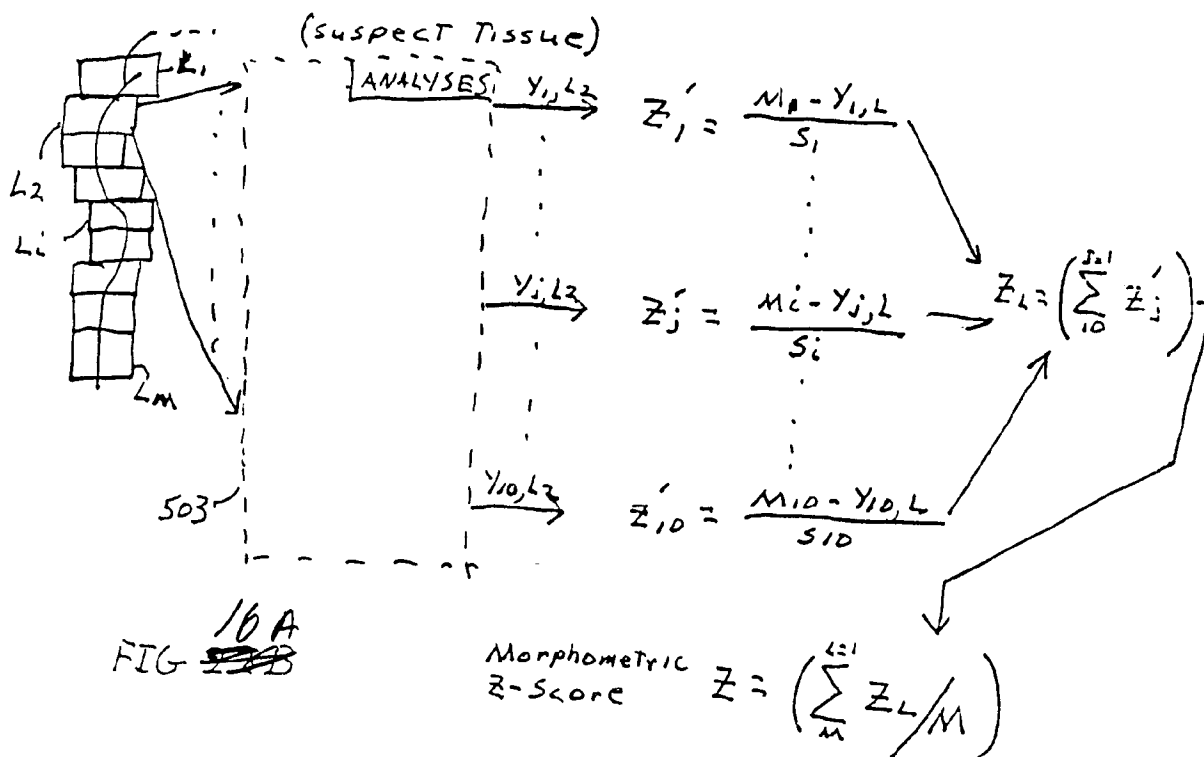
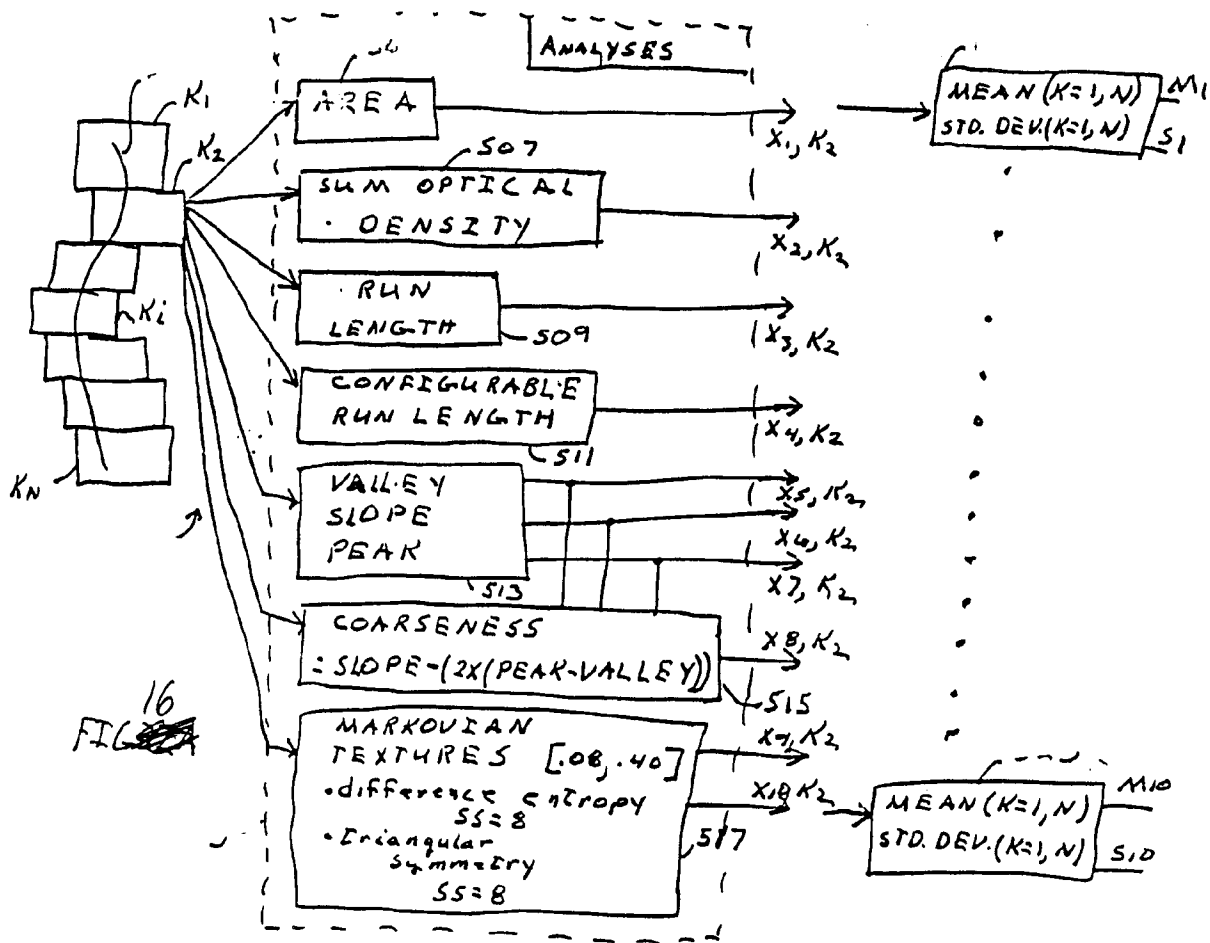


~~FIG 20D~~  
Fig. 15A



~~FIG 20C~~  
15

14/15



15/15

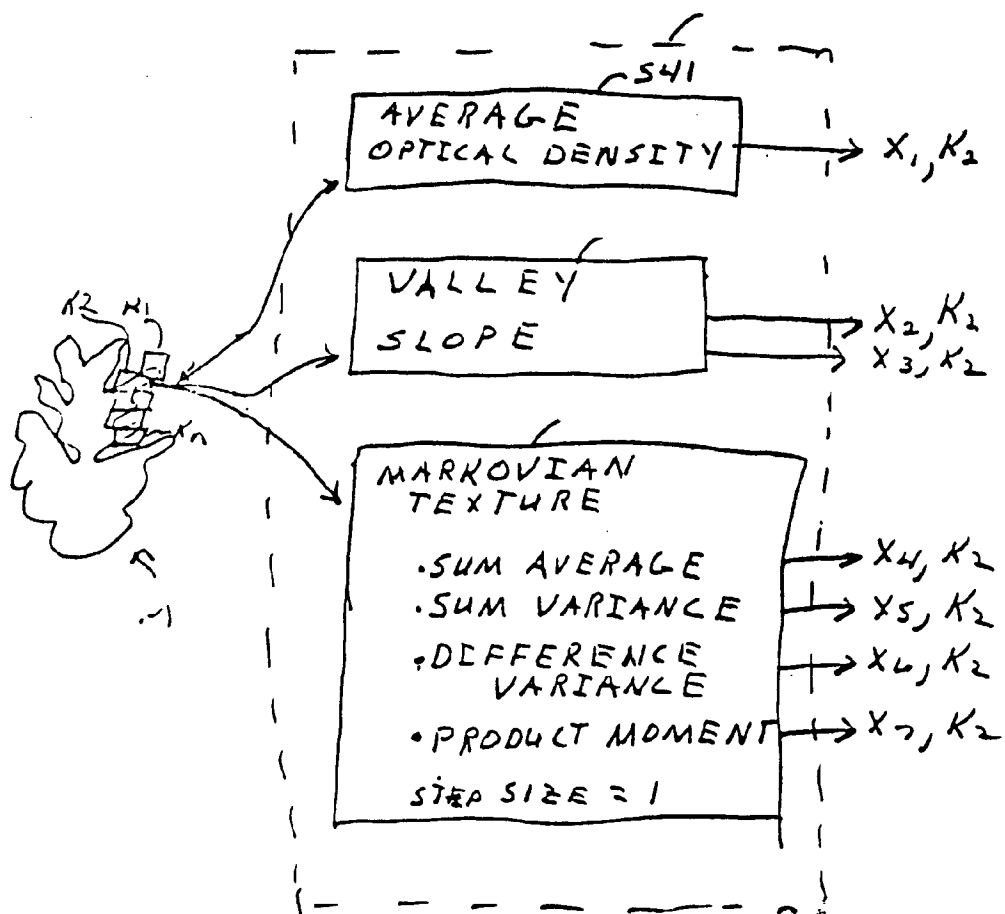


FIG 23 17

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/04208

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :G06K 9/00

US CL :382/133

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 382/128, 133, 134, 284, 294, 318, 319; 128/922

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,257,182 A (LUCK et al) 26 October 1993, Figures 1 & 2; column 3, line 23 through column 5, line 12; column 7, line 1 through column 8, line 5.	1-46
Y	US 4,287,272 A (RUTENBERG et al) 15 February 1994, Figure 3A; column 4, lines 39-62; column 12, lines 19-56; column 13, lines 27-61.	1-46
A	US 5,544,650 A (BOON et al) 13 August 1996, Figure 2; column 4, lines 32-65.	1-46
A	US 5,428,690 A (BACUS et al) 27 June 1995, column 3, lines 12-38.	1-46
A	US 5,252,487 A (BACUS et al) 12 October 1993, see the entire document.	1-46



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

15 JUNE 1998

Date of mailing of the international search report

09 SEP 1998

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

ANDREW W. JOHNS

Telephone No. (703) 305-3900